Elementary Microscopical Manipulation

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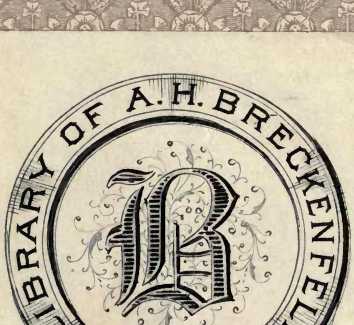
T. Charters White, F.R.M.S.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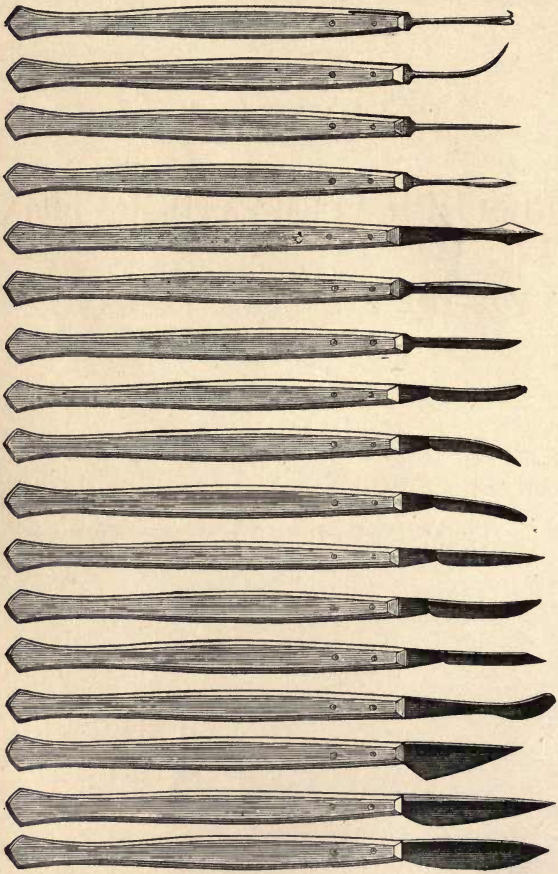

Dissecting Knives.

A MANUAL

OF

ELEMENTARY

MICROSCOPICAL MANIPULATION,

FOR THE USE OF AMATEURS,

BY

T. CHARTERS WHITE, M.R.C.S., L.D.S., F.R.M.S.

Late President of the Quekett Microscopical Club.

London:

ROPER & DROWLEY; 29, LUDGATE HILL, E.C.

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INTRODUCTION.

This small Manual is not intended to supersede the larger and more extensive works dealing with the Microscope and its revelations, and which treat in the fullest manner of all that appertains to various instruments and the optical principles involved in their construction. Neither does it profess to teach the natural history or minute anatomy of the various subjects included in the immense range of microscopical observation. For instruction in these, the student must be referred to the bibliography in the Appendix.

It is designed with the aim of affording the youngest beginner such directions for preparing objects of interest and instruction in an elementary, but at the same time such a complete manner that, be he the merest tyro, he may grasp their details and work out his studies with the most satisfactory results; hence this work may be regarded as but a Manual of Elementary Microscopical Manipulation for those possessing a microscope and desiring to prepare and mount their own specimens.

32, Belgrave Road, S.W.,
November, 1887.

ELEMENTARY

MICROSCOPICAL MANIPULATION.

Microscopical observation has received a powerful impetus within the last few years by the introduction of really good, cheap, and powerful instruments. As optical science has improved and competition between makers has been keener, so the microscopes of the present day have in their essential points been made so excellent as to leave little to add or desire. It is now within the power of any one able to command a few pounds sterling, and having the desire to investigate the marvellous microcosm lying around, to procure such an instrument at a moderate cost, as will give correct defining powers, a good field of view, and a magnification of from 30 to 300 diameters, with all the necessary stability in the microscope stand which constitutes comfort in working.

An interest has awakened amongst all classes of late years in the minute structures of animal and vegetable life. The subject has been brought under the notice of the public by lecturers, as well as by frequent reference to it in the columns of the press, and probably many might take up this most interesting study were the imaginary obstacles removed from the path of its approach. It is the *raison d'etre* of this little Manual to deal with these difficulties in the most explicit manner, and thus lead the possessor of his first microscope into the smooth path of progress, by pointing out the simplest and most elementary methods of observation, and after so far clearing the way leading him gradually to the higher branches of Microscopical Manipulation.

THE MICROSCOPE.

Microscopes may be divided into two classes—simple and compound. The simple microscope may be exemplified by the ordinary hand magnifier, generally consisting of one lens or a set of three, which may be used singly or in conjunction. A simple microscope of this class is useful, and should be the pocket companion of every microscopist; not only will it be found of value in the examination of any object which may casually offer its attractions in his walks in the country, but will also aid in his recognition of infusorial forms in his pond explorations; it will also furnish a most valuable adjunct in mounting objects as well as in insect dissection. But for more extended observation, recourse must be had to the second class—the compound microscope. The essential parts of this may require a word of explanation. Firstly, the objective, or as it is sometimes called, the object glass, is the lens or series of lenses placed at that end of the microscope tube next to the object to be examined. It is generally compounded of several lenses of varying curvature, and differing refractive powers, cemented together and securely adjusted in a brass mount. When successfully mounted, they should give clear definition, a flat field, and entire freedom from colour, all of which characters may be found in the achromatic objectives sold by our good makers. Objectives are of different focal lengths, ranging from four inches from the object examined to one-fiftieth of an inch. The lenses sold with the student's microscopes are usually one inch focus, magnifying fifty diameters, and a quarter of an inch giving 250 diameters. These have been most judiciously selected for the beginner as giving amplifications which will be found sufficient for all ordinary work he may be likely to attempt at first. The object having

been magnified by this lens, its image is received by the eye-piece, so named from being placed at the upper end of the microscope tube next the eye of the observer, and where it undergoes a further amplification. The microscope tube is mounted on a stand, and the focal adjustments are made either by moving the tube up and down through a cloth-lined tube, through which the microscope is made to slide, or by a rack and pinion movement, which is the more convenient method.

Below the stage, on which objects are placed for examination, is suspended a mirror, having plain and concave silvered surfaces, by which daylight, or that from a lamp is transmitted through the object. Between this and the stage, a revolving disc of metal, termed the diaphragm, and having apertures of different sizes, is fixed. This enables the observer to regulate the amount of light received by the transparent object, in such a degree as to reveal details in it, which otherwise might be lost in a flood of unnecessary light. The student having a microscope of this plain and simple description, needs but a condensing lens, either attached to the stage or separately mounted on a foot for proper lighting of opaque objects, to have enough to enable him to begin his attack on the world of wonders lying at his feet. Before, however, he does so, a few directions as to his method of working may not be out of place.

Lighting the Object.—The light employed for microscopical examination may be either the light from a good paraffin lamp, or daylight. Of these, the latter is preferred, as less fatiguing to the eyes, besides giving a more correct view of the object examined. The light from a white cloud in the northern sky is especially recommended, but where this condition is unattainable, a piece of white paper placed on the mirror furnishes a very good substitute. As the student may not have the opportunity during daylight for his investigations, he must perforce use lamplight, and by placing a lump of camphor about

the size of a walnut into the reservoir of the lamp, and burning some well purified paraffin such as Strange's Crystal Oil, a brilliant white light can be obtained, which may, at any time, be softened by reflection from paper.

The lamp should be placed at the observer's left hand, and in advance of the microscope; it is not then in the way of his right hand and any apparatus placed there for use.

SIMPLE APPARATUS.

THE student in microscopical observation should begin his investigations by the study of the simplest subjects. Unfortunately the tendency of most newly interested observers is to rush into an examination of the higher and more complex objects before they are thoroughly versed in the appearance presented by those elements of which the higher classes are constructed. It is well, then, if the student can restrain his ardour till he has acquired familiarity with such simple objects as the hairs of various animals, fibres of cotton and wool, the starches of wheat, potato, and rice, and such like subjects which may be found ready to hand. These may be followed by the examination of the several elementary tissues entering into the formation of the animal and vegetable structures.

When he has familiarised himself with these comparatively simple objects, he may then pass on, with profit, to the examination of the higher structures, when his preliminary training will be turned to advantage in the ready recognition of many of his early acquaintances amongst the histological elements. Such a course as this tends to a gradually increasing interest, while the reverse generally ends in a settled indifference, because the

evolution of a natural interest has been stifled by a premature attempt to mentally grasp what only a trained eye can comprehend.

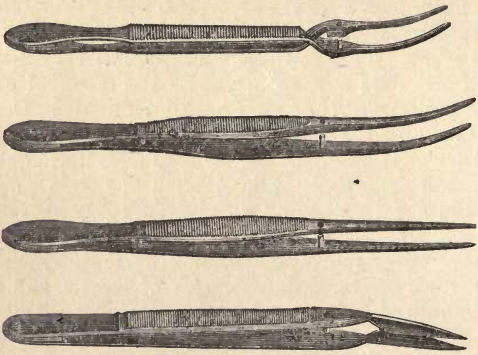
Before the student commences the work of his microscopical observations, it is necessary that he should provide himself with a few indispensable appliances; to assist him, a brief enumeration may save trouble:—

Glass Slides.—Before observing any transparent object under the microscope, it must be supported on the stage of the instrument on a glass slide beneath the objective. These glass slips, procurable of any optician, are of a standard size of three inches long, by one inch wide. They should be of patent plate, free from bubbles and striæ, and ground at the edges. They are not expensive, as they may be cleaned and used over again should the mounted object not be satisfactory. If they have been used for Canada balsam mounting, a short immersion in methylated spirit, to which some liquor potassæ has been added, will quickly remove the mounting medium, when the slide may be washed in warm water, and wiped clean, ready for further use.

If the object under examination be opaque, such as seeds, pollen, grains of flowers, eggs of insects, and such like, the rays of light may be concentrated on them by the condensing lens, which is sufficient preparation; indeed, a juster idea of the nature of the object may be formed by this method than by any other; but if the object is intended to be examined by transmitted light reflected from the mirror beneath the stage, then it is desirable to place the object under a covering glass to keep it flat.

Cover Glasses.—These, also obtainable at the opticians, are of differing degrees of thinness, varying from one-fiftieth to one three-hundredth of an inch in thickness. Unless the magnifying power employed be greater than one-sixth of an inch focal length, it is advisable for the

novice to use the thicker kinds, as the thinner glass for use with the higher powers of the microscope breaks so readily in cleaning, and it is very necessary that cover glasses should be absolutely clean. Cover glasses are square or circular as the exigencies of the object may demand, or the fancy of the student may desire. Some use square covers for everything; but the circular covers have so many advantages, that they are to be preferred. These will be referred to at a later period, when treating of mounting in cells and in fluid. When these cover glasses come from the opticians, they are not clean: but before

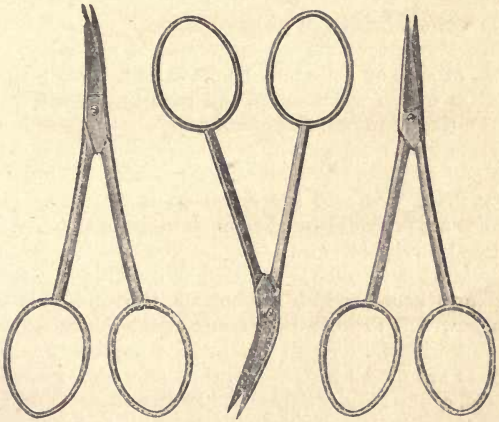


FORCEPS.

using they must be scrupulously cleaned, by steeping them in warm spirit and water, and, with very delicate handling, wiped dry with an old, soft silk handkerchief, and stored away in a clean box till they are required for use. A simple but very safe apparatus for cleaning the very thin glass without breakage, is formed by having two blocks of boxwood, having their ends turned perfectly flat, and covered with smooth buff-leather, upon which has been rubbed a little putty powder. These surfaces being smooth

and perfectly flat, the thin glass may be rubbed between without the chance of its being fractured, when a slight dusting with the old handkerchief suffices to remove any stray particles of dust.

Forceps.—The cover glasses after cleaning are best kept free from stains, by being handled with forceps; this plan also enables the operator to lay the cover glass over the object without disturbing its surface so much as if clumsily laid down by the fingers. Forceps are of two forms, curved and straight, both being found very useful in the preparation and mounting of microscopical objects.



SCISSORS.

Scissors.—Two pairs of these will be found of great service in the preparation of microscopical subjects. They should be small and delicately made, and kept beautifully bright and free from rust. One pair should be straight, while the other ought to be curved sideways, but both coming to fine points. Another pair may be added to

these, but much smaller and more pointed with a spring between the handles to keep the blades apart when not in use. This will be an indispensable advantage in the dissection of insects, full directions for which will be given in the section devoted to that subject.

Needles.—These are useful in laying out membranous or other thin subjects, so that they may be as free from creases as possible, or for removing stray particles of foreign matter, which, if allowed to remain, would detract from the cleanness and beauty of a mounted preparation. They may be made very easily by drilling holes in wooden penholders, and inserting ordinary needles, some may have a stout bristle in place of a needle—needles may be either straight or curved—where it is necessary to use acids, needles of glass may be made by melting a thin rod of glass over a spirit lamp, and pulling out a fibre of it of the size required, this glass needle will be found very suitable where chloride of gold is employed as a staining fluid, as metal needles cause a deposit of the gold and a dirty stain wherever the specimen is touched. If steel needles are prepared—and some descriptions will be found of great service later on—they may be coated with gold by shaking up an aqueous solution of chloride of gold with sulphuric ether, which taking up the gold from the solution, will deposit it on any steel which comes in contact with it. When the needles are broad and flat, such as are used by surgeons, they may be mounted in a handle, ground and sharpened, highly polished, and gilded with this solution, they then form excellent scalpels for the minute dissections of insects, and the gold coating prevents their contracting rust, which catching in the delicate fibres of a partly dissected insect, would spoil several hours' work.

Wash Bottle.—This is a very useful appliance in many microscopical operations, it is readily made by taking a wide-mouthed well-corked four-ounce bottle, then perforate

the cork in two places, pushing through two tightly fitting glass tubes, that one reaching to the bottom of the bottle may be softened at a point just above the cork by holding it in the flame of a spirit lamp, and bent to an acute angle of 70°, its outer end melted and drawn to a point which may be chipped off according to the dimensions of the exit required. The second tube, which will constitute the mouth-piece, may be pushed only just through the cork, and its outer part bent to an obtuse angle of 120°. By partly filling this bottle with distilled water and blowing through the mouthpiece, the pressure of the air on the surface of the water forces it through the other tube, and it may be directed in a fine stream, but with considerable force against any section or other substance it is desirable to wash. Similar bottles may be made to hold glycerine, salt solution, or any of the mounting fluids to be treated of later on.

Watch Glasses.—The student is recommended to get about half a dozen of these; they are used for soaking specimens in staining fluids, and for holding isolated specimens. There are some glass pots having lids about the same size as watch glasses, which are very handy and inexpensive.

Wine Glasses which have become broken from their stems, are useful for placing over preparations which may be soaking in spirit or staining fluid, if the operator is called off or has to leave his work for something, as they ward off dust and check evaporation.

Dipping Tubes and *Stirring Rods* may be obtained of any chemist, and cannot be done without.

It is sometimes necessary in warming slides, when mounting in Canada balsam, or in making troughs with marine glue, to hold them over a spirit lamp. It is not comfortable to do this at the risk of burnt fingers, and therefore this may be done more safely and easily by holding the slide in a pair of wooden forceps constructed as

follows:—Take two strips of deal of about equal length, and thin them off at one end, between the thick ends, glue in a small block of wood, and, if necessary, tack the slips to make them more secure. This will be found a useful accessory for this purpose, and, moreover, the glass is not so likely to fly with the heat as if held in metallic forceps.

Having furnished himself with these indispensable appliances, the student may proceed to make such simple investigations as his immediate surroundings may furnish. The more simple and elementary they are, the more sure will be his progress. An examination of such an easily obtainable subject as a drop of saliva will afford very instructive lessons in the flaky masses of flattened epithelial scales filling the field of view. The immense number of round dark rings are airbubbles—which once seen in this fluid, will be readily recognised whenever met with afterwards. The drop of saliva being placed on the slide, the cover glass is gently picked up in the forceps by one edge, while the opposite side is lowered on to the fluid, and allowed to fall by its own weight. All fluid mountings should be accomplished in this manner.

PRELIMINARY EXAMINATIONS.

Microscopical subjects may be divided into opaque and transparent, both sub-divisions teeming with interest, but requiring different treatment in their examination. The examination of opaque objects requiring less preparation than those of the transparent sub-division, may well occupy our consideration in the first place. Having a glass slide on which the opaque object is placed, it is only necessary to throw the rays of light on it with the condensing bulls-eye lens supplied with the microscope, and

examine it first with a low power, and when all its general appearance is mastered, then using a higher power lens to investigate the detail of its exterior. We are not able in the examination of this class of object to do more than gain a knowledge of its external characters; nevertheless the student will find abundant material in it to interest and instruct. It may save him trouble and be suggestive, if he commences his observations with such as may be found in the following list, viz., minute animals, such as cheese and other mites (*acari*). *Poduræ*—little silvery animals generally found abundantly in fern cases and amongst moss, or under damp stones or tiles in the garden, where they may often be found in company with a little light brown pseudo-scorpion called *chelifer*. In the examination of this class of object, it is very necessary to employ a *cell*. A cell is anything which can imprison and confine, and a microscopical cell may be constructed of any material by which you can form walls either circular or square, to be roofed in with a cover-glass, for by this means you are enabled to restrain the peregrinations of your menagerie. If many chelifers are confined together, they will quickly mutilate each other, and even one kept in a cell has been known in its futile rage and despair to tear off its own pincers, or its legs. As the *arachnida* to which they belong include on the one hand the scorpions, and on the other the spiders, we may naturally look for something rapacious in their habits, minute as they may be—these are but few amongst many instances which might be given—but it will be sufficient to indicate these few as suggesting the line of observation the student may take with advantage. Whole insects or parts of them may be submitted to examination. One suggestion may be in the direction of looking at the eyes of flies of various kinds, or their mouths or proboscis, and it affords a very interesting study to imprison a blue-bottle fly in a small paper cone, the apex of which has been cut off

sufficiently to allow the fly to pass its head and proboscis through, when it is generally fixed, and not easily withdrawn till the cone is undone. This method will exhibit in an interesting manner the working of the proboscis or so-called blow-fly's tongue, the contraction and expansion of its disc showing the horny chisel-shaped teeth which lie on either side just within it. A live flea may be put under a wine-glass, on some blotting-paper, with a few drops of chloroform. The flea being rendered insensible, may be affixed by a minute drop of gum on its side to a white card. By the time the action of the intoxicant has passed off, the gum will be dry, and the drunkard will find herself in a fix, and in her endeavours to walk home, will display the action of the legs and oral apparatus. These are but suggestions to direct the ingenious student to devise other ways and means for observing the various parts of living insects. One of the most ingenious as well as most useful appliances to be used in connection with opaque living objects is Mr. S. J. McIntire's cork cell, which is constructed in the following manner:—Take a slip of cork, such as employed to pin insects out in museums, cut it to the dimensions of the microscopical slides, then cut an oval hole, with slightly tapering sides, through its centre, and about one inch by three-quarters of an inch, place glass sides under and over the cork, interposing a pad of wet blotting-paper between the bottom glass and the cork, and bind all together with two elastic rings. Any such small animals as the poduræ and their congeners kept in these cells in the damp and fed on oatmeal, will thrive and multiply, besides being always ready for examination when wanted; thus their life history may be traced and their habits watched. It would absorb our space to too great an extent to treat exhaustively of the numerous opaque objects of interest which the student can readily avail himself of, and which abound on all sides.

MOUNTING OPAQUE OBJECTS.

The student may be desirous of mounting some of these opaque objects for future reference or investigation, and, to preserve them, they must be mounted in cells of a suitable depth—microscopical cells are square, oblong, or circular—according to the shape and size of the object to be preserved. If the square, or oblong cell is chosen, slips of glass of a suitable thickness, and rather more than one-hth of an inch in breadth, may be procured from any glazier; with short pieces of this the cells may be built, the sides being affixed to a glass slide by warm marine glue; when cold, the superfluous marine glue may be cleaned away, and the bottom of the cell obscured with a black varnish, such as Brunswick black, or it may be painted with Indian ink, as it is desirable that no light should be transmitted through the cell in examining opaque objects. When the cell is thus far constructed, it should be set aside to become thoroughly dry, or a most annoying dew settles on the inside of the cover glass, rendering the object indistinct. The author has often found it useful, in these cases, to leave the cover glass temporarily fastened down, by tacking it in two or three places with some wax, with which a small quantity of Canada balsam has been incorporated, to make it more adhesive. The cover then is sufficiently fastened for safety, at the same time excluding dust, while it can be removed at any time should it require cleaning. This mixture of wax and Canada balsam will be found extremely useful in many ways later on, and several ounces might be prepared in readiness for future use. In mounting very thin opaque objects, which do not need a cell of any depth, it is sufficient to cover them with a circular glass, and by means of

a smooth, round piece of iron or brass rod made hot, to run some of this wax preparation round in the angle formed by the edge of the cover glass and the slide, when, on touching the cold glass, it sets and seals the edge, allowing of a coating of varnish to be immediately placed on, for greater security and permanence, without the danger of its running in and spoiling the object.

Subjects requiring but a slight addition of depth beyond that sufficient for the last class of objects, may be mounted in a varnish cell. A ring of some hard varnish, such as that made of gum dammar, dissolved in benzole, is made on a glass slide by means of a "Shadbolt Turntable," and when sufficiently hard, the object may be mounted within it. The cover glass being chosen rather smaller than the ring, may then be fastened on with the wax and Canada balsam before a permanent cement is put on, or there is great danger of the cement being drawn in by capillary attraction, and the preparation spoiled. Neat glass cells may be constructed in the following manner:—Take a brass plate, having circular apertures in it of the sizes the cells are required to be, and with marine glue cement a square of thin glass over one of the selected holes. When the glue is quite hard, give a smart puncture in the centre of glass with a small rat's-tail file, when the glass will be starred, the cracks, however, not proceeding beyond the edge of the aperture. The glass may then be filed out under water to the edge of the aperture. Great care must be exercised afterwards, in warming and removing the glass from the plate, though, if a simple fracture should occur, it is of no great consequence, as the cell joins again readily when cemented to the glass slide.

It may sometimes be useful to cut a hole through the slide itself, which may be accomplished in this fashion:—Take a hard steel drill, having a spear-headed point, dip it in turpentine, and drill partly through the surface of the slide at the point opposite to that in which the cell is

to be made; then attack the other surface, so that the hole made shall correspond to that already begun, and by the application of fresh supplies of turpentine, continue the drilling till the point comes out at that first commenced, when the drill comes through without cracking the glass or starring its surface. Having got the hole, it may be enlarged by the use of the rat's-tail file under water to the dimensions required. Where it is desirable to examine test objects through thin glass, this may be cemented over such a hole, and the test object mounted on it in the ordinary way, and covered with a similar piece of cover glass.

Glass rings, corresponding in dimensions with the diameters of the most generally employed circular covering glasses, are exceedingly valuable for mounting opaque objects. They may be cemented to the glass slide with marine glue; their surfaces being left rough from the cutting, afford a firm attachment for the glue, which diminishes the risk of the ring coming off the slide with too energetic wiping. Rings of pure tin or zinc can also be obtained at any of the dealers in microscopical materials, and are useful from the ease with which they can be reduced in depth by filing, should they be too deep for the object to be mounted. Rings of gutta-percha, vulcanite, and millboard, have found favour with some; but their qualifications for making a sound and secure mount have been so deficient, that they have been discarded by all good mounters. Brass rings are not suitable, as the copper in them stains the mounting medium green after the preparation has been set up a short time. There are some cells termed ground-out cells, which can be highly recommended to those who mount small insects as transparent objects; they may be had of different sizes, to suit different subjects. They are, as their name implies, ground out from the surface of the slide, and afterwards polished. For mounting those objects which show their points of interest to the

greatest advantage without pressure, they are most satisfactory. Having treated of those cells most usually adopted, we are now ready to pass on to the various cements employed, both for fastening the cells to the slide as well as the cover glass to the cells.

Marine Glue.—Nothing can surpass the utility this cement presents, whether in the tenacity with which it holds, or the readiness with which cells or troughs for the examination of aquatic life can be constructed; it is the most suitable and convenient the microscopist can employ, as, by its aid, he can join glass and metal in any variety of form his requirements may suggest. To melt it, gentle warmth must be used, but the temperature must not be sufficient to burn it, or its adhesive properties are destroyed. However valuable it may be for the purposes here indicated, it is not adapted for cementing cover glasses. For that we require a varnish which can be put on without heat, and of this kind we have a variety to choose from. Whatever varnish we use as a cement, must not be too thin. It ought to be of the consistency of syrup, or that of the densest and freshest glycerine. If it is firmer than that, there is a difficulty in sealing up a preparation so that the joint is solid and free from leaks. If it is thinner than that, there is the danger of its running in; but if it is about the firmness indicated, these liabilities are greatly lessened, if not entirely abolished.

Gold Size.—This is by far the best cement for this purpose, for a cement should not become hard too quickly. It is better that it should take a day or two after a first *thin* application, before it is sufficiently set as to allow of a second and thicker coating. This cement answers well for sealing up cells containing any of the preservative fluids, but especially for those not containing glycerine. Glycerine is influenced very greatly by changes of temperature and barometric pressure. It is also hygroscopic, therefore moisture is readily absorbed by it; so, if the cement is

cracked by the expansion of the glycerine, moisture is soon absorbed, and swelling the fluid contents of the cell, causes a general bursting of the cement. The author has found the value in glycerine mounting, and with other preservative fluids as well, of an addition to the gold size of about one-third solution of India-rubber in bezole. This gives an elasticity to the gold size, which seems to allow of a small amount of expansion without cracking of the cement. The older the gold size, the more reliable is it as a cement.

Gum Dammar Varnish.—This is a clear, tenacious cement, which may be readily made of any consistency desirable. By putting clear pieces of the gum into benzole, and, when dissolved, letting the benzole evaporate till the fluid is of the density required, it may be decanted off into small bottles for use. It dries very readily, and a thin coating put round a cell is sufficiently hard in a couple of hours to receive an additional one. There are several other cements more or less recommended in books; but the student will find in these two all that he needs to meet his requirements. We now pass on to a consideration of the various preservative media employed.

MOUNTING TRANSPARENT OBJECTS.

Preservative Media.—These are of two kinds—the solid, such as Canada balsam and glycerine jelly; and the fluid, as glycerine, Goadby's solution, and such like.

Canada balsam, while undoubtedly that which takes the first place, especially with novices in the art of mounting, on account of the apparent simplicity in its use and the readiness with which a slide can be prepared by its means, has its drawbacks; and perhaps the first which the

student will experience, is that arising from the presence of air bubbles; but this liability can be overcome by placing the object to be mounted on a small portion of Canada balsam, previously put on the slide, and then dropping another small portion on the centre of the cover glass, and upon bringing the two carefully together with the aid of the forceps, the air is excluded. While Canada balsam is an extremely useful agent in various degrees of age and hardness as a mounting medium, it will be found more useful if thoroughly hardened and redissolved. Take it as supplied by the dealers, and pour it into a glass, and evaporate it by heat, till, upon dropping a little into cold water, it is found to set hard like a piece of resin; it may then be set aside to cool, when it is ready for solution. Two solvents have been recommended: chloroform and benzole; of these two the author prefers the latter, and advises its use, because after solution in this it becomes very limpid and colourless, any foreign matter in it soon settling to the bottom; it should be kept for use in a capped bottle, and if after a time it should become stiffer, it may be diluted with a little benzole. One great advantage of this solution is the readiness with which air bubbles in it disappear after a few hours. The slide and preparation may be plentifully covered with them, but they soon become absorbed, and finally the preparation is quite free from them.

Glycerine Jelly is a very uncertain preparation, and after a time develops large air spaces in it, probably the result of desiccation; it necessitates the object to be mounted being previously well soaked in glycerine. It is very liable to air bubbles in its use, which are difficult to get rid of by reason of the tenacious character of the gelatine.

While Canada balsam and glycerine jelly readily lend themselves to the mounting of such preparations as are substantial in their character, as are the sections of dif-

ferent tissues, hard and soft; there are other preparations as dissections of insect anatomy, and delicate objects, both animal and vegetable, which would be crushed out of all recognisable shape by the pressure necessary in using such comparatively hard mountants as these; therefore recourse is had to fluid preservative media, to which attention may now be directed. In using these, it is well to bear in mind the necessity of considering the density of the fluid medium, that it bear a proportionate relation to that of the preparation to be mounted; for instance, a delicate object, especially if of a tubular character, would collapse and be crumpled out of its proper shape, and probably spoiled, by being placed in dense glycerine, but by altering the density of this by dilution the character of the preparation can be preserved. Thus glycerine is a favourite mountant from its adaptability to almost all subjects, and the facility with which it unites with other chemicals; nevertheless, it needs discrimination in its use, as it cannot be employed for calcareous tissues as bone or shell, as they would become decalcified after being exposed to its influence for some time; therefore for these preparations only balsam should be used. Glycerine used in its full strength is highly refractive, and is thus useful in bringing into view the minute details of many structures. Objects to be preserved in dense glycerine should first be soaked in a diluted solution, and passed through solutions of increasing density, till that of the original glycerine is attained; in this way all risk of distortion is averted. Glycerine diluted with camphor water makes an excellent preservative fluid, but one which finds much favour amongst microscopists, and is useful for the majority of preparations, is made from a mixture of glycerine one part, water two parts, and alcohol three parts, commonly known as the one-two-three mixture. If it is desired to render an object transparent, it may be soaked in this mixture, and lightly covered up to protect it from

dust, when the alcohol will evaporate, leaving the glycerine and water in the tissues.

There is one disagreeable drawback in glycerine as a preservative, and that is the difficulty of cementing it in securely. It needs great care and scrupulous cleanliness in its use; but this may, to a great extent, be acquired by employing as frequently as possible the same size of covering-glass, when, by practice, the amount of glycerine which will be sufficient to flow to its edges without extending beyond, may be accurately judged. A cement may then be applied, which will keep the glycerine in. If any flows beyond the edge, it may be blotted up with bibulous paper, and cleaned away; but it is difficult to make any cement stick to the slide securely when it has been once wetted with glycerine. Several plans have been suggested and adopted to overcome this difficulty; one of which seems to answer fairly well. It consists in drying up the glycerine as thoroughly as possible, and painting round the edge of the cover a thin layer of glycerine jelly. When this has set, any of the usual cements may be laid over it. A cement to be used for glycerine mounting, and which is said to be very easily applied, and to stand very permanently, is made as follows:—Carbonate of lead, two drachms; red oxide of lead, two drachms; litharge, three drachms, to be well mixed and levigated. When wanted for use, mix sufficient for the mounting with a little gold size, and apply at once. This cement is also spirit proof. There are many plans suggested for overcoming this difficulty, but it is of no great use to the student to overburden this manual with them. The employment of these two, and the mixture of gold size and india-rubber solution given on a preceding page, will be found amply sufficient to meet all demands.

There are other preservative media, which, after glycerine, are comparatively easy of employment, such as alcohol and distilled water, camphor water, syrup, &c., which may now claim our attention.

Alcohol is well known as a preservative, but it has so great a tendency to render delicate tissues opaque by coagulation, that for microscopical mounting purposes, it is never used in its full strength. A preservative fluid should interfere as little as possible with the normal characters of a preparation, and should be what histologists designate an *indifferent* fluid. Some beginners might think that distilled water ought to answer to this character, but it does not, and should only be used for the most transient examination. Let him prove this statement by the examination of blood corpuscles in water, and he will soon see them changed into every shape but their normal one. That water may be used, for even a casual examination it is necessary that it should be combined with some other substance that will give it a specific gravity equal to the liquids contained in the tissue under examination. For this purpose, a weak solution of common salt in water is employed, and answers fairly well. It gives its best effects if a little gum arabic is dissolved with it. A stock solution of it may be made by dissolving one drachm of common salt in a pint of distilled water, and adding four grains of gum arabic; this is then always ready for use, should immediate examination be necessary. This forms a very fair and stable mounting medium, so that in the event of the subject under examination proving of interest, it will be only necessary to let the fluid be dried up round the edge of the cover glass, when it may be cemented with gold size or stiff gum dammar varnish.

Another useful fluid mountant will be found in a nearly saturated solution of acetate of potash. It is found valuable in the mounting of vegetable preparations. The preparation being placed on the glass slide under the cover, this solution may be added drop by drop till the intervening space is filled, when it may be sealed in with cement. Its refractive powers are not equal to glycerine, but in cases where glycerine is not admissible, this will be found an excellent substitute.

Preservative fluids vary according to the substance to be mounted, whether it be vegetable or animal, whether the animal be marine or freshwater, and even different parts of the animal body require their suitable medium. Therefore any notice of them to be of value to the student, would be precluded here, from want of space; but a reference to works in which fuller details may be found, will obviate the necessity of swelling the dimensions and cost of this small production.

Whatever mounting medium be employed, nothing conduces more to success than neatness in manipulation and regularity in arrangement, and mounted specimens add considerably to the pleasure of keeping a cabinet if all the slides are correctly labelled, and no preparation should be put aside without a rough label stuck on *pro tem.* If the student intends returning to it for further observation at some future time, it is hopeless to say it will be remembered. A month or two after, a doubt will arise in the mind, and six months after, its source, name, its very nature are involved in obscure forgetfulness, it may then be thrown away, for its usefulness as an instructive agent has vanished. A simple plan when preparing a series of slides, is to have some of the gummed bordering from postage stamps, rolled on a reel and hung up near the mounting table, and when a slide is put up, and before placing it in the cabinet, to stick on a temporary label from this roll and pencil the name of the subject, and any particulars relative to its mounting medium, &c., as may be needful. Not only should the slides be labelled, but it adds considerably to their neat appearance if the mounts are in the centre of the slide. It is not sometimes an easy matter, especially with delicate or fragile subjects, to get them into this desired position, but, by gentle coaxing, and if a thin section, by judicious floating on to the slide, it may be made to occupy the centre. There are, however, some subjects whose constitution is suffi-

ciently robust to stand a good deal of pushing about, where a small piece of apparatus used by the author for some years with comfort, may be adopted with great advantage, whereby the subject may be placed centrally with the greatest facility—a thin plate of wood covered with white paper, and rather larger than an ordinary standard slide, has a wooden ledge glued along one side, and shorter ones at the ends, enclosing an area which will just receive the glass slide on which the subject is to be be mounted; if two lines are drawn diagonally across this space from the opposite corners, their intersection will be the exact centre of the slide. Taking this intersection as the centre, circles corresponding to the size of the circular covering glasses usually employed, may be struck with a compass, and in this way, objects mounted within the circles seen through the glass slide must be centrally placed. A very ingenious plan of mounting, devised by Mr. J. Guardia, may be introduced here. It is desirable sometimes to be able to examine an object from both sides. It may be thus accomplished: procure two thin strips of wood of the standard size, from the centre of one, cut out a square three-quarters of an inch side, from the other cut out a square seven-eights of an inch side, glue the two strips together, and it leaves a ledge for the preparations to rest on. Specimens, therefore, if mounted on glass covers seven-eights of an inch square, drop into this frame or carrier, and can be turned and examined from each side.

Having thus far briefly touched upon a few of those preliminary details which are necessary for elementary microscopical observation, it is time to pass on to some of those preparatory processes by which it may be further advanced. The student, after having examined such objects as may be readily obtained, and which have required no special preparation, may pass on to those needing some treatment to reduce them to a condition suitable for investigation.

SECTION CUTTING.

Probably the first desire, as it is also the more easily gratified, is that of being able to make sections. Tissues to be reduced to sections come under two heads—hard or soft. As each requires a different treatment, they must be dealt with separately; sections of hard tissues being more readily accomplished, will command our first attention. The hard tissues comprise such substances as bone, teeth, woody shells, such as cocoanut shells, the various hard woods, and stems of plants. There are two methods by which sections of osseous tissues may be obtained: they may be made by sawing off thin slices and grinding them down to the requisite thinness, or they may be soaked for several days in weak acid and water till the lime is dissolved out of them, when thin sections may be cut with a razor. The former method may be adopted, not only from its simplicity, but because it will give a more correct insight into the histological characters of the bone, than is afforded by the softened and dislocated elements of a decalcified section.

As it is not the intention of this Manual to treat of the histological characters, information on which point must be sought after in the works referred to in the concluding bibliography, it will be convenient to describe the method usually employed for

Grinding down sections of bone and teeth.—The piece of bone to be cut having been previously freed from grease in a solution of common washing soda, is cut into slices with a watch spring saw; in this condition the slice is too thick, and little or nothing of its histological structure can be seen. It may now be rubbed down between two plates of ground glass, with the addition of some pumice powder

and water, when by grinding one plate upon the other, the slice of bone between gets gradually thinner, till, if the process is continued without great care and frequent examination, the section disappears altogether; as the pieces of ground glass get rubbed smoother by use, it is better if we wish to avoid such a mishap as this, to change the section to some of this older and worn ground glass, when it will not be ground down so quickly at this stage, and will be moreover polished by the smooth glass, which is very desirable if a good mount is cared for. The smoother pieces of glass when wet, allow the process of grinding to be watched very narrowly, and stopped at the right time. There is another advantage in using two plates of ground glass, and that is, in the sides of the section being ground quite parallel throughout the process: it is by far cleaner than the methods of grinding sections usually given in books on microscopic manipulation, and is as applicable to woody shells of fruit, and teeth, as it is to the osseous tissues. If sections of teeth with the enamel *in situ* are desired, a slight variation must be made from the method just detailed.

Corundum hones, of varying degrees of fineness, may be obtained at many tool shops. The corundum, an imperfect form of ruby, having its hardness, but not its colour, is ground fine, incorporated with melted shellac, and moulded into hones, files, and wheels. Such a hone is a valuable accessory in this process. Sections of teeth are difficult to make, on account of the excessive hardness of the enamel, a file making no perceptible impression on it if used dry, but wetted with water, solution of soft soap, or turpentine, it can be attacked. But those who attempt to cut it, even by these means, will find the saw, so usually recommended, but a poor tool against the enamel, and will break many saws before getting through it to the softer tissue beneath. A lapidary's wheel is the only weapon with which many slices of a tooth can be

cut, the number being governed by the thickness of the wheel; but teeth can always be rubbed down on one side on a wetted corundum hone, or, what is better, a corundum wheel attached to a lathe, where it is only desired to make one section. Having ground the tooth down vertically to near its middle, it may be cemented, with very little heat, to a piece of glass by some very old and hard Canada balsam, and then the other side ground down as the first had been. When ground to the thickness of a card, it may be detached from the glass, and further reduced to its final thinness between the plates of ground glass; but this method, while satisfactory so far as the dentinal tissue is concerned, is apt to leave the edges of the enamel chipped, frayed, and presenting ragged edges. This may be obviated by grinding the section to the middle as before, then polishing the surface very highly with a wet buff leather charged with putty powder. When this surface has received the highest polish it is capable of taking, it may be cemented as before with hard balsam, taking particular care not to heat the glass slide to a greater degree than will be sufficient to soften the balsam; for if made too hot, the polished surface of the section will be spoiled, presenting somewhat the appearance of a china plate which a neglectful cook has made too hot in the oven; an appearance not to be admired in a section under the microscope. Having, therefore, attached the tooth firmly and in closest contact with the slide, leaving support of the balsam round its edge, grind down the section to the utmost thinness, and then highly polish it, as done on the previous surface.

There will be then a thin polished section only needing to be permanently mounted, a proceeding which requires some care, and to which it is necessary to devote some space if the work of hours is to result in a successful termination. Most of these hard tissues, whether of bone, teeth, or nut shell, have an internal structure of great

interest, whether viewed with high or low powers. Thus in bone there are the lacunæ, with their exceedingly fine system of ramifications, in teeth the almost similar lacunæ of the cementum, the dentinal tubuli, and the fibres of the enamel; and it becomes a matter of necessity to mount the sections obtained from them, in such a manner and by such methods, as while preserving the specimen, shall not obliterate the structure; the examination of which was the main purpose of the section. It is found that if a section of either of these substances is put directly into balsam, the air in the internal cells becomes absorbed by the balsam, which running in to fill the vacuum, reduces the histological detail to the dead level of one uniform transparency, and all evidence of the structure is lost. Means must therefore be adopted by which this disadvantage may be obviated, and two plans are offered, either of which entirely prevents this destruction of detail.

The one the author has adopted for several years with satisfaction and success, may be first described. It is obvious that in all structures having internal cells, the fluid media in which they may be mounted will, by endosmosis, run into them, and therefore means must be adopted of keeping this medium out. Thus Canada balsam, used to fasten the tooth to the slide in grinding the section, must not be warmed to any such extent as would liquify it; neither must it be dissolved off in such menstrua as chloroform or benzole, but it may be removed by soaking in absolute alcohol, which, softening the Canada balsam without dissolving it, enables the section to be removed, after which the Canada balsam may be cleared away with a camel's-hair pencil; when perfectly clean, it should be transferred to clean distilled water, and allowed to remain till the spirit in the spaces has given place to the water—it may then be carefully dried on its surfaces, and mounted in balsam of a moderate

degree of stiffness, when the water in the tubular structure will prevent the balsam running in and obliterating it.

Another plan, by which the same results may be brought about, was introduced to the author by Mr. J. E. Ady. He says: "I have lately been experimenting, with a view to the effective mounting of bone, teeth, and other similar structures in Canada balsam, and am glad to say that I have succeeded beyond my expectations. I adopted three methods for the occlusion of the minute spaces, lacunæ and canaliculi in bone, and the dentinal tubuli and interglobular spaces in teeth, prior to mounting the sections in balsam. 1st.—Collodion film answers very well, but is troublesome, and sometimes fails. 2nd.—Mucilage film strained B. P. solution of pale acacia), answers perfectly, but is apt to harbour dust, unless extreme care is taken. 3rd.—The laccic method—coat with an alcoholic solution of *white* shellac. This is practically the best and simplest method, provided the slide is not heated to over melting point of shellac; a mishap which none but a slovenly mounter would incur."

The finished sections, when ready for mounting, are dipped momentarily in the shellac solution, and withdrawn when a thin coating of the lac is left over its surface, occluding the spaces, but not running in. Instructive sections may be made of many structures which contain loose parts, by allowing them, after dehdydrating in absolute alcohol, to soak in a thin solution of balsam in benzole till it has permeated all the parts, when the specimen may be removed, and set aside to evaporate and harden; thus corals, teeth in jaws of small mammalia and such-like, may be rubbed down thin without fear of dislocating their integral constituents.

Sections of Echinus spines are amongst the most interesting objects of study, but on account of their friable nature some of the most difficult to make. This difficulty can be almost overcome by soaking the spine in Canada

balsam dissolved in benzole, and if it is previously steeped in the benzole, and then placed in the solution of balsam, it runs into the structure, thus supporting it during the grinding. This may be greatly facilitated by afterwards placing it in a gentle heat, such as a slow oven, till the balsam becomes hard. There is no difficulty in cutting the section with a fine piercing saw moistened in spirit, when the section may then be rubbed down to the necessary thinness on a hone, the balsam dissolved out in benzole, and the section mounted in the solution of Canada balsam.

Section-cutting also includes that of the soft tissues, which may now demand our attention. As it is desirable sometimes to make sections of the hard tissues, in conjunction with the soft parts attached to them, and as it is not possible to grind sections of them in the manner just described, recourse is had to decalcification, by which the lime is removed, and the bony tissue rendered soft enough to cut with a razor. There are various solutions of the acids by which this process may be carried out, but the author has found the readiest and most efficient agent for this purpose in a saturated solution of common alum to which has been added a few drops of hydrochloric acid to each ounce of solution; while in this mixture the acid dissolves the lime, the alum hardens the soft tissues, so that, in a short time, it is possible to cut very thin sections, showing all the relations between the soft and hard elements of a structure—such sections requiring only very profuse washing, to free them from the chemical before they are ready for staining and mounting. There are other substances from which sections may be made, occupying an intermediate position between the hard and soft tissues, such as hair and cartilage, and it will be desirable to treat of them here, before going on to the preparation of sections from soft tissues.

Hairs are horny cylindrical structures springing from a

papilla situated at the end of a tubular depression of the skin, and in investigating its histology, these two structures, viz.: the hair and its follicle should be studied in conjunction. This can be done by sections taken in two directions, and the use of suitable reagents. There are two methods by which horizontal sections of hair may be made—one being to arrange a bundle of hairs longitudinally, and soak it in glue; when this has set, the bundle may be cut in thin slices with the microtome, the glue dissolved, and the sections picked out, and mounted in Canada balsam. The other plan is to shave the beard very closely with a keen razor, and after a few hours, to shave again; in this manner very thin horizontal sections of hair, and a very fair sprinkling of oblique sections may be easily obtained. Good sections of this kind may be so readily purchased of the preparers, that it is scarcely worth the trouble entailed in making them. Vertical sections through the hair follicles, showing the structure of the root of the hair, may also be purchased; but in many cases the anatomical details are so obliterated in the mounting, that it becomes advisable to make such if we would see the relations of the hair to its follicle and its surrounding histological elements.

Portions of the scalp, or such other hairy parts of an animal as may afford the requisite depth of substance for the follicle, may be cut into small cuboid portions placed in ammonium chromate, and examined from day to day till sufficiently firm to cut into sections; choosing then a piece in which the line of section would correspond with direction of the roots of the hairs, a section should be produced which would show all we needed. Fairly good specimens of the roots of hair may sometimes be obtained by slowly drawing out that hair of the beard, which may generally be found occupying the centre of a pimple, the inflammation and consequent effusion of fluid into the surrounding tissue so loosens the root of the hair, that it

may be readily detached, bringing with it parts of its sheath and the cells. These preparations, after staining with carmine, show their histological elements at their best when mounted in glycerine.

Cartilage is another of those semi-hard substances which occupy an intermediate position between the hard and soft tissues, requiring no preparation before cutting, and may be dealt with at this stage. This substance is commonly known under the name of "gristle," a thin section under the microscope shows it to be be made up of nucleated cells, distributed through a semi-transparent solid mass. Thin sections of this cartilage are best obtained by free hand-cutting with a razor; in examining these some indifferent fluid should be used, as pure water quickly produces a change in the form of the cells. The fluid which answers better than many is a solution of one part of chromic acid to six hundred of water, and should the preparation be deemed worthy of preservation, it can be sealed up in this, when it will maintain its characters almost for an indefinite time.

Cartilage presents itself under different characters; thus in that which may be examined in a section from the "gristle" of a shoulder-blade of mutton the cells are irregularly disseminated throughout the mass; while in ossifying cartilage from the joint of a young or fœtal animal the cartilage commences in the usual manner, but soon a disposition evinces itself towards an arrangement of the cells in lines coincident with the axis of the long bone on which it is found. Very interesting is the variety found in the mouse's ear, where it resembles chain armour in its appearance; to obtain this the ear must be macerated in water till the skin softens by decomposition, when it may be peeled off the cartilage. In examining cartilage, much of its histological detail will be lost from a full illumination by direct transmitted light; it is, therefore, advisable to tilt the mirror slightly, in order to modify the intense light

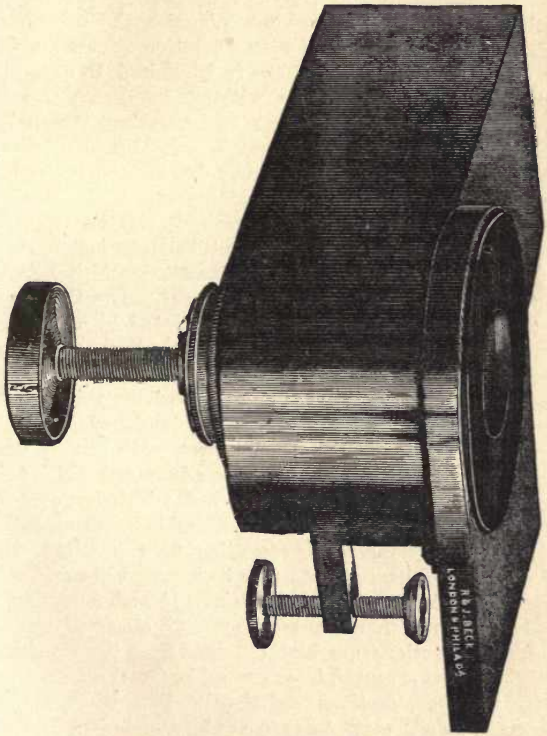
which will be more than necessary, when the cellular structure will be distinctly seen.

These are not the only conditions in which cartilage may may be met with, but are quoted as indications for its easy examination in cases likely to be met with by an ordinary observer; for this substance may be found in reptiles and fish as well as in the mammalia, and its character being once attentively studied, can be readily recognised again.

The student may now wish to examine in section some of the soft tissues, and may commence by cutting sections by free-hand cutting; he will find it convenient to begin upon such vegetable substances as are firm to hold, and yet soft enough to yield very thin sections. The substance to be cut must be held firmly by the fingers and thumb of the left hand, the knuckle of the forefinger being raised as a guide as well as a rest for the razor, by which means the thickness of the slice may be regulated; when the beginner can cut thin and even slices of vegetable tissue by this method, it will enable him to so hold and cut similar sections of the animal tissues with equal facility. Many good histologists prefer this mode of cutting sections to that which needs a microtome, and practice, in their case, seems to make perfect, as far as sections of moderate tenuity are concerned; but no one who has once experienced the comfort of a microtome will care to go through the drudgery of learning to cut sections free-handed, and therefore the student is recommended to get one, if his researches are likely to lead him into the engaging field of histological investigation.

There are many and various forms of microtome: from the elaborate and beautiful instrument of the Cambridge Physiological laboratory, to the simple but efficient instrument of Professor Stirling's; but the student will probably content himself at first with such as the latter with which to try his prentice hand. The principle upon which they are all constructed is either that by which the substance to be

cut remains fixed, while the moving razor is lowered by the agency of a screw, having a very fine thread, as in Williams' freezing microtome, or, the substance being



PROFESSOR RUTHERFORD'S MICROTOME.

raised by infinitesimal degrees by a similar screw, whilst the fixed razor traverses it, and slices off the sections; it

will therefore be seen that the thickness of the section depends upon the fineness of the threads of the screw, and the number of degrees it is rotated.

A good instrument for this purpose is known as Rutherford's freezing microtome, in which the substance to be cut is first saturated with a solution of gum arabic, and then frozen; in cases where it is desired to examine sections from recent tissue without having to put it through the preliminary stages of hardening, it can be adopted with great success, and it can also be used for hardened tissue; therefore, it may be recommended for a good, cheap, all-round instrument, and steady, when, by means of its screw-clamp, it can be securely fastened to the work table. For a still simpler instrument, nothing is better than Ranvier's microtome, which is held in the hand while the sections are cut. The thickness of the sections is, as in others, regulated by a thumb-screw at its base, by which the paraffin plug, containing the embedded material to be cut, is made to protrude from the upper end, according to the extent of the turn of the screw, the razor being supported on a circular plate, which surrounds the top, being kept wet with methylated alcohol, slices off the section. One advantage which this form of the instrument supplies, and which is not possessed by others, is this: if it is desirable to examine the first few sections before proceeding to cut others, the microtome and its contents may be inverted in spirit, and kept moist till it is wanted again. It also furnishes a ready means of cutting sections from stems of plants. Hardwood sections are best obtained from the shavings, which may be procured from any cabinet-maker.

In this way, sections sufficiently thin to show the structure well, may be procured. Good longitudinal sections of hardwoods, as walnut, teak, mahogany, and cedar, when mounted in Canada balsam, afford instructive and interesting specimens for the cabinet. Fossil woods

being silicified, can only be cut by a lapidary's wheel. These and sections of coal, which may be placed under the head of vegetable sections, are better purchased from those dealers who provide them, for coal-cutting is dirty work, and troublesome withal; but should the student feel inclined to try for himself, he will find full particulars relative to the *modus operandi* in Dr. Carpenter's "Microscope and its Revelations."

The animal tissues in their recent condition are too soft to admit of thin sections being cut from them; they have therefore to go through a preliminary hardening process, to which we may now devote a short space. There are various hardening agents, and it will be necessary to discuss their respective capabilities and advantages. One of the readiest is absolute alcohol. This agent being anhydrous, soon abstracts all the water from a tissue, and hardens it ready for cutting in about twenty-four hours; but its action is so powerful, that while it hardens rapidly, it causes an undue shrinkage, and thus may be the means of misleading the student into false ideas of the nature and histological characters of his section. It is better that the small piece of tissue to be cut should be immersed in a large quantity of some aqueous solution containing chemicals, which have the power of coagulating its albuminous elements, and fortunately we have a number from which to choose. A two to five per cent. solution of bichromate of ammonium, or of potash, used in considerable bulk, compared with the size of the mass of tissue to be hardened, works as satisfactorily as any solution the author has tried. Chromic acid, in strengths of one-fifth per cent. to one-half per cent., may also be recommended; but if all risk of shrinkage is to be avoided, it is better to begin with a weak solution, and gradually increase its strength. Müller's solution is also valuable in many cases. It is made by adding two and a half parts of bichromate of potash, and one part of sulphate of soda, to

one hundred parts of water; but unless the student intends going in for extended histological research, the ammonium bichromate will be amply sufficient; and if he wishes to proceed further, information will be found in those works quoted in the appendix.

In whatever manner, or by whatever means, sections are cut, one thing is absolutely necessary, and that is the possession of a very sharp, hollow-ground razor, kept for this purpose only. Great care must be taken that its keenness is maintained by frequent stropping on a firm flat strop; indeed, sections will be obtained with greater success if it is stropped after every two or three cuts, and when finished with for the day, it should be stropped again and put away clean. Many are very careless in this respect, but it is a mistake to let it get dirty and spotted with rust, when it ought to be so smooth that the almost cobweb-like sections it cuts should slip over its surface freely. If spots of rust are allowed to roughen this surface, the sections are frequently caught in them and torn. The next step in section cutting is *embedding*. The hardened tissue, cut into blocks of a square form, should have its surfaces dried with clean blotting paper. If it is the intention of the microscopist to cut it by the free-hand method, he should proceed as follows:—A piece of thin lead foil, or a piece of writing paper, should be rolled round one end of a ruler and the end beaten in to form a sort of round case; or a piece of paper, or, what is better, thin card may be folded up into an oblong dish of sufficient depth to hold the tissue to be cut, leaving plenty of space around it. Pierce the tissue with a fine needle, and run its point through the paper dish into the table, taking care that by this means the block of tissue is supported in the required position; then melt some paraffin wax, to which a little lard has been added, and directly it becomes fluid, and before getting too hot, pour it into the tray, when it will embed the object to be cut. The embedding mass should, when cold, always bear a relative

hardness to the embedded tissue, and it can be modified by the addition of lard, to approximate to the condition of the substance to be cut; or paraffin wax of different melting points, and varying hardness, can be selected for this purpose. When the mass is hard, it may be placed in spirit (the lead foil or paper being previously removed), till required. If a microtome is used, the melted embedding mass may be poured into its well, and while it is soft, the tissue inserted in the desired position and held there, till fixed by the cooling of the mass. There are other methods of embedding, such as steeping the subject in gum, and afterwards hardening the gum by immersion in spirit. There is also the plan of filling cavernous tissue, such as the lung, &c., with melted cacao butter, before embedding in paraffin, but they are omitted here, to limit the bulk of this volume. Full directions for the use of these will be found in those books quoted. We have now only reached the preliminary stages of hardening and embedding; we will now proceed to the cutting of the sections. If the student has practised free-hand section cutting, he may take the mass in his hand, wrapped round with a piece of blotting paper, and, holding it firmly, steadily cut away the mass in thin slices, till it reaches the tissue, when he may take a slice off; this will not be kept, as it is merely to give a smooth surface to start from. The razor must be kept well wetted with spirit, and drawn with a decided cut from one side of the tissue to the other; any pause in the cut resulting in an ugly line across the section. At first there may be a difficulty, but after a little practice a clean, sweeping cut will be attained, the section being so thin, that every mark on the razor can be seen through it. These thin slices, as they are cut, may be floated into a watch-glass of spirit, where, if sufficiently thin, they may remain awaiting the next process, while the mass may be returned to the spirit in the event of its being wanted again.

After cutting very thin sections with the microtome, a

trouble very frequently arises in transferring them from one solution to another as in staining and clearing, by their doubling up, and getting entangled to their damage and detriment. This may be avoided by adopting the section lifter recommended by Dr. Sylvester Marsh. This useful piece of apparatus may be made by bending a narrow band of German silver about half an inch in width, so that its lower end forms a flat blade at a convenient angle for passing under a section, and lifting it out of the different fluids it may have to pass through.

STAINING TISSUES.

STAINING has added a fresh power to the microscope, for without its aid, the highest magnification would fail to show many interesting details. Staining may be general or selective; by the first, nothing is learnt, but it is useful sometimes in showing up the structures, which are too transparent to be seen otherwise, and is of great service when the object is to be photographed, in presenting a contrast against the general field of view. By selective staining, which selects the growing parts of a tissue, the nuclei are brought into view, and the formed material is differentiated from that which is in process of formation. The sections may now be stained. Some histologists prefer to stain the hardened tissue before cutting, which is followed by very good results; but staining the section is just as easily done without requiring so long an immersion as staining it in the mass. The stains can be procured all ready prepared, at any of the chemists supplying histologists; but there are one or two which are made without any difficulty, and which the student can readily concoct for his own use, they are carmine, logwood, and picric acid. Judson's dyes also furnish a handy supply, and possess that

permanence which should be the character of all these stains. Dr. Beale furnishes such plain directions for making the carmine stain, that it will be of service to insert them here:—

"Carmine	10 grains.
Strong liquor ammonia ..	½ drachm.
Price's glycerine	2 ounces.
Distilled water	2 „
Alcohol	½ ounce.

"The carmine in small fragments is to be placed in a test tube, and the ammonia added to it. By agitation, and with the aid of the heat of a spirit lamp, the carmine is soon dissolved. The ammonical solution is to be boiled for a few seconds, and then allowed to cool. After the lapse of an hour, much of the excess of ammonia will have escaped. The glycerine and water may then be added, and the whole passed through a filter or allowed to stand for some time, and the perfectly clear supernatant fluid poured off and kept for use. This solution will keep for months; but sometimes a little carmine will be deposited owing to the escape of ammonia, in which case one or two drops of liquor ammonia to the four ounces of carmine solution may be added."

Logwood stain may be made so easily from any of the numerous formula given in the text books, that it may seem unnecessary to unduly burden this small Manual by giving one; the desire of the author being to furnish the student with as much elementary information as he can, which, while condensed, will yet be clear and serviceable to him.

"Take Logwood extract	6 parts.
Alum	6 „
Sulphate of copper	1 part.
Water	40 parts.

"All ingredients must be free from iron.

"Grind the alum, logwood extract, and sulphate of copper in a mortar, and when powdered, add sufficient

water to form a thin paste, leave for one or two days, with occasional stirring, and then filter."

The author is indebted to Mr. A. B. Lee's "Microtomist's Vade Mecum" for this recipe, and having some years ago made the stain from a similar recipe, he found it work very efficiently, at the same time being so easily made, he can recommend it.

Picric acid, which has the property of staining yellow such structures as the epithetial surfaces, requires but solution in distilled water to fit it for use. Sometimes it is used in combination with carmine solution; but it is preferable to use them separately or conjointly as cases may require. The sections which have been cut may be placed in either of these as the student may choose, but he should bear in mind the advantage of using these stains diluted, and of slowly staining them. For this purpose, watch glasses or shallow glass dishes will be found of great use. The sections being placed in the staining solution in one of these may be covered up and left for a short interval, till the staining is considered deep enough, when it may be removed to clean distilled water, and examined; any stray fragments being removed by brushing with a camel's hair brush kept for that purpose. If the section is sufficiently stained, it may be transferred to a small quantity of absolute alcohol to dehydrate it. Having been steeped in this a short time, put a drop of oil of cloves on a glass slide, and taking up the section with a pair of forceps, drain it from the spirit, and when nearly dry, place it on the top of the oil of cloves, which, thus taking the place of the spirit, as it evaporates, will clear the section and render it transparent. If the section is left too long in the oil of cloves, it becomes shrunken and distorted; therefore as soon as it is quite evenly clear, it may be drained from the oil and mounted in balsam, perferably the balsam and benzole solution. This, then, is the *modus operandi* to be observed in all section mounting, subject to varying treatment when other mounting media are em-

ployed, and for this the student must be referred to the bibliography.

While on the subject of staining, it may be interesting to direct the beginners' attention to the beauty of some vegetable sections, if double stained, and as this does not entail much difficulty in its accomplishment, it will be an addition to his pleasure if directions are here given whereby he can make them.

Judson's scarlet dye may be added to water till a good colour is obtained, when the solution must be filtered through blotting paper. Judson's blue dye may be dissolved in methylated alcohol, and the solution filtered. These solutions, well corked, keep for a considerable time. Prepare two watch-glasses, and put a little of the red solution in one, in which place the section. When it becomes stained an uniform red, remove it to the other watch-glass, in which pour some of the blue. This will drive out the red stain from all but those definite portions of the section to which the red will cling with great tenacity; after which, it may be treated according to the foregoing directions, and mounted permanently.

Public attention has been frequently directed to the subject of bacilli, and their occurrence in the sputa of phthisical patients, and a chapter devoted to staining would be incomplete without reference to a method of staining bacilli, which seems to give good results all round. The sputum is spread in a thin layer on a cover-glass, and dried at a temperature of 212°-230°, or passed three or four times through the flame of a spirit lamp. The staining fluid is prepared as follows: 5 c.c. pure aniline added to 100 c.c. of distilled water, well shaken, and then filtered through moistened blotting paper. To this mixture a saturated alcoholic solution of fuchsine, methyl violet, or gentian violet is added, till precipitation commences. The cover glass is allowed to float on this with the sputum downwards for from a quarter to half hour. It is then washed for a few seconds in nitric acid, one part, distilled

water, two parts, and afterwards in distilled water alone. It may then be dried and mounted in balsam.

Chloride of gold is employed as a staining agent when it is desirable to bring out the exceedingly fine ramifications of nerve tissues, but care is required in its use to avoid overstaining. The solution should be very dilute, about one part of the gold salt to two hundred of distilled water. The section may be placed in this from a quarter to half-an-hour, and then removed, freely washed with a large quantity of distilled water, to which a sufficient quantity of acetic acid has been added, to render the water perceptibly acid, and placed in the light, which, acting on the gold, gradually darkens it to a pale violet colour. If the gold solution has been too strong, or the immersion too prolonged, the section will become of such a dark purple as to be almost useless.

Diluted solution of osmic acid is useful for staining fat cells; but great care must be taken by the student in avoiding the fumes, which are very injurious to the eyes and mucous membranes of the nose and throat. The fumes themselves are sufficient to stain many delicate preparations and even to kill small insects.

It is useful to be able, in many cases, to identify starch cells, and the stain or reagent which may employed is:—

Iodine	..	..	.. 1 gramme.
Iodide of Potassium			.. 2 grammes.
Water	..	..	.. 100 c.c.

A small quantity of this placed upon starch will turn it at once to a purple colour, and proves its nature beyond doubt.

It is sometimes necessary in preparing vegetable sections to bleach them prior to their undergoing staining processes; for this purpose they may be placed in a solution of chlorinated soda, readily purchased of any chemist. This removes all the green colouring matter harmlessly, and renders them, after copious washing, ready to take any stain intended.

INSECT ANATOMY.

THE student may now desire to advance a step further, and if he has followed out the previous directions carefully, he may have acquired that dexterity and steadiness of hand which will be required in that very fascinating pursuit—the study of insect anatomy. No great difficulty will obtrude itself in his path, but a great draft will be made on his patience, for nothing can be successfully accomplished in this department without great care and an entire absence of hurry. It is more conducive to ultimate success if some of the larger insects are chosen to begin upon, and such an easily procurable object as the common kitchen cockroach will furnish a convenient, and, at the same time, most interesting "subject" on which to commence operations. In all investigations into the structure of living animals, the humane student will shrink from giving unnecessary pain, even to the smallest of created beings, and should always remember that, in depriving them of that life which he cannot confer, it should be done as painlessly as possible under the influence of an anæsthetic. Chloroform offers a safe, and, at the same time, the least painful agent for killing insects. The insect to be dissected should be placed on a piece of blotting paper under a wine-glass, with about thirty drops of chloroform, when in a minute's time it will have been killed without apparent suffering, and operations may be commenced. Before proceeding to the dissection it will be well, in all cases, to form a definite plan, and to make up the mind to the particular part to be investigated; and, as it has been the aim of these instructions to offer to the student the simplest methods at first, it may be as well here to recommend the abdominal viscera as presenting the least difficulty. The plan to be adopted in this, as in all cases,

is first to remove with scissors such embarrassing organs as the legs, wings, and antennæ; the insect may then be securely fastened below water, to which a few drops of alcohol has been added—the alcohol slightly hardening the delicate viscera, while the water floats them, and keeps them from becoming unduly entangled. There are two methods by which this very necessary security may be attained; that most generally recommended is by pinning the insect to a layer of entomological cork, attached to some sheet lead; but a plan which the author has adopted with great comfort and convenience, is as follows:—Take a piece of gutta percha, such as supplied for boot soles, cut a piece four-and-a-half by two-and-a-half inches, soften it in hot water, and bend up its edges into a dish, drop on to the bottom of this some of the wax and Canada balsam mixture, and while it is warm and soft stick the back of the insect into it; in this way we get rid of the pins, which, under the old plan often get in the way. When the composition has set, and the insect has been ascertained to be securely stuck down, the water and spirit may be poured into the dish, and the dissection commenced. This may be done under either a regular dissecting microscope, or by such ingenious arrangement as that devised by the late Mr. W. T. Loy. This simple piece of apparatus may be constructed by the student in this manner: a stout wire of brass or iron should have one end fastened into a heavy square foot of lead, the other end of the wire must be bent into a ring, which will hold a watchmaker's eye-glass. The trough containing the insect should then be mounted on a block of wood to bring it within the focus of the glass. In carrying out the dissection, the eye is placed in contact with the circular frame of the eye-glass, and by raising or lowering the head, the focus is adjusted, the weight of the leaden foot keeping it in position, and allowing a movement up or down as the exigencies of the dissection demand.

Many operators hold the glass in their orbit as the watchmakers do, but this involves so much muscular fatigue, that the plan recommended will be found more agreeable, and the results eminently satisfactory, a low power objective, such as an inch and a half focus may also be employed, but the constricted field of view does not compensate for the higher magnification. If the student can afford a Stephenson's Binocular erecting microscope, this is the acme of perfection and convenience, and by its means and a steady hand the author has often dissected out the salivary glands of a flea—the possession of this instrument may be characterised as the height of luxury—but simpler weapons, suffice for very good work. Having this or any similar arrangement, with the scissors, cut up the brown chitinous skin on each side of the abdomen, taking care that their points do not penetrate so deep as to injure the internal organs, then with the forceps raise the skin, beginning at the thoracic end, and as it is raised clear away the tracheal attachments by the aid of perfectly bright needles, free from all traces of rust. It is here the gilded needles will be found so advantageous, for these delicate tracheæ cling with great tenacity to any roughness, and although at this stage of the proceedings it would not be of much consequence, at a later period such a catastrophe as the entanglement of the tracheal tubes in a rusty spot would be fatal to success, and spoil the work of hours.

If the brain and neural system are to be dissected, it will be found more convenient to place the insect in the wax composition on its ventral surface, dissecting from the dorsum. Any organ which may be deemed worthy of preservation may be detached with scissors, and after staining, may be mounted in diluted glycerine. It is advantageous to have a knowledge of the names and uses of the various organs, and a reference to the works of Burmeister and Lowne, quoted in the appendix—may

furnish information relative to some of these. It is one of the disadvantages of the present state of entomological science that the fullest information could be obtained, but it is scattered all up and down in the walks of literature, instead of being gathered under one compilation. This, then, is, in brief, the *modus operandi* to be pursued in all insect dissections, which, if the student adopts, will afford him unspeakable interest in enabling him to compare the variations of the organs of different subjects; take for instance those peculiar glandular bodies found in the rectal pouch of various insects, the rectal papillæ. In the blow-fly they are four in number, of a pyramidal shape, projecting from the walls of the rectum, where they perform the functions usually carried on by the kidneys in the higher animals, secreting uric acid, well formed crystals being frequently found in the pouch. These same organs in the earwig are six in number, of a circular shape; in the flea they are six in number, but oval, in the bee they are eight in number, and elongated. These few instances are given, to show the abundant field of interest awaiting the industrious and intelligent worker in this department of biological science. It may be of service to state here that many interesting points may be made out in the examination of newly hatched insects; for instance, the wings of a butterfly are far more beautiful objects if the butterfly is removed from the chrysalis before it had time to spread its wings, so in the blow-fly freshly hatched from *gentles*, many parts, such as the tongue, can be more easily mounted in the orthodox fashion than after they are allowed to get old and stiff. A freshly hatched blow-fly may be fed on cochineal and sugar, when on dissecting the alimentary canal, it will be found coloured with the carmine for its whole track, and made more evident in the dissection.

Many interesting and beautiful specimens may be made from small insects which have been steeped in the strongest

carbolic acid solution, especially if they are to be mounted entire without compression. Calvert's crystallised carbolic acid, which is solid at ordinary temperatures, is the most suitable; if this is liquified by heat, and about one drachm of glycerine added to about two ounces of melted carbolic acid, it will prevent its becoming solid again; and in this condition it is not only useful for rendering insects transparent, but can be used for clearing all stained vegetable sections instead of oil of cloves, if so desired. It may sometimes be necessary to bleach insects, especially if they are to be photographed through the microscope, which process will be detailed later on. Such a bleach may be made by placing some crystals of chlorate of potash at the bottom of a wide-mouthed two-ounce bottle, and pouring on one ounce of water; if to this a few drops of hydrochloric acid be added, chlorine gas is slowly evolved, which, acting on the chitine of the insect's skin, bleaches it after a week's immersion as white as paper. It also must be observed that it destroys all internal anatomy by its corrosive action, and the student must be careful not to inhale the fumes from it, as bronchial mischief may easily ensue upon an incautious breathing of this gas. Interesting specimens may be made from the corneæ of insects, the larger kinds, such as the *Dytiscus marginalis*, furnish the best, as they are more easily removed, and are tougher to manipulate. On cutting them off they will be found convexo-concave in shape, the concavity being filled with pigment, this must be carefully cleansed with a camels-hair brush. They are difficult to mount, on account of their convex form; but by judicious slitting in two or three places round the edge and by pressure, they may be flattened sufficiently to show the lenticular structure. If properly prepared, each facet shows clearly any object placed on the mirror beneath the stage, thus, small portraits, the seconds dial of a watch, &c., are reproduced in each lens, and afford much interest. The recent eyes of insects are

also interesting as opaque objects, but soon lose their beauty on account of *post mortem* changes, which render them black. Opaque objects of rare beauty, as are some of the green weevils, may be got by sweeping a bed of nettles with a bag-net of muslin; these keep their brilliant colour for years. They should be mounted in a rather deep glass ring cell, the bottom and sides of which have been painted with a dead black varnish; when this is thoroughly dry, the weevil may then be set upright on its legs, the abdomen being attached to the bottom of the cell by a small portion of the wax and Canada balsam cement; this will form a good and tenacious attachment, the legs may be arranged in their natural positions, and the cell covered with a circular glass cover, just tacked in two or three places with the wax and Canada balsam. This will keep the cover safe, and should it become dewed by any exhalation either from the varnish or the body of the insect, it is readily removed, wiped, and replaced. It is always a good plan to mount opaque objects after this manner.

Not the least interesting study for the young microscopist, is that which may be devoted to that branch of insect life which comprises the parasites of different birds and animals. Here they may be found in infinite variety, every host being the entertainer of strange and wondrous forms of life, and as these are obtained with great facility, no difficulty should be experienced in making a collection. A reference to Denny's Monograph, included in our appendix, will afford some slight insight into the abundance of parasites infesting animal life, all of which can be readily mounted in balsam, after a preliminary clearing in carbolic acid. To obtain them, it is only necessary to take a canary cage infested with "lice," when an extensive gathering may be made of the *Dermanyssus Avium*; or examine a domestic fowl, and numbers of its parasites can be got—pigeons are fruitful hunting grounds. If a bird

or a small animal is killed, and whilst warm is placed on a sheet of white paper, its parasites will begin to leave directly the body cools, and may then be readily collected. The Acaridæ or mites present an infinite variety of beautiful forms, which an examination of the splendid Monograph published by the Ray Society will testify. Mr. A. D. Michael, in this charming monument of his many years' labour and observation, shows what can be accomplished by the painstaking student in this, almost the smallest class of created beings. The student is to be envied the first glimpses he gets of these mites; the old observer has seen them and similar objects, and time does not stale the interest, but yet he is inclined to look back to that period when all these were fresh to him, and burst on his gaze as a new revelation

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INJECTION.

When the student has advanced so far in this course, he may undertake the injection of the blood-vessels of such small mammalia as the rat, or guinea-pig, with a view to the demonstration of their circulating system. As this is an operation of some difficulty, requiring much care, a knowledge of anatomy, even if it is but elementary in its character, will greatly assist him; therefore, in laying down directions for his procedure, it will be well to direct his attention, in the plainest manner possible, to the structures and organs met with in commencing operations. Injections of the circulating system of such small animals as those above quoted, and which form the best subjects for the beginner, are more easily done from the left side of the heart, and, to reach this, the directions generally

given are to make a longitudinal incision through the *sternum* or breast-bone, and stretch the opening sufficiently wide to get to the heart. This plan of commencing is very embarrassing, and affords but small space for the delicate manipulation required at the most critical part of the operation. It is recommended, through a fear of the injecting-fluid escaping through the intercostal arteries which run along the course of the ribs; but, in a small animal, these are not of sufficient size to make any material loss, certainly not one to be considered when the advantage of gaining more room is in view; therefore, it is better to make an incision transversely across the lower part of the thorax, about the distance of six ribs from the *clavicle*, or collar-bone, then, with a stout pair of scissors, cut through the ribs each side, and remove the front of the thorax in one piece—the lungs are then seen apparently filling the whole cavity of the chest; but, on pressing them apart in the centre, the heart, in its pericardial bag, will be found. The *pericardium* may be opened and removed, as may also the greater part of the lungs, unless it is desired to inject them; but till the student is able to carry out this operation with that perfection which comes of practice, it is better that he should limit his labours to the simple and less-demanding process of injecting the arterial system. The apex of the heart may be cut off with scissors, when two openings in it will be noticed—these are *the ventricles*—if a probe be carefully inserted into the lower of the two, that nearest to the vertebral column, it may be made to pass through the valves which guard the entrance to the *aorta*, or main artery of the system into the arch of the aorta, which bends over before it gives off its ascending and descending branches. The greatest care must be exercised in performing this operation in a small animal, as the tissues are delicate, and rough manipulation would end in a rupture, which would spoil the injection. Having said so much by

way of a slight and rapid glance at the anatomy concerned in this process, we may pass on to the formulæ recommended for the injecting fluids.

Injections may be either transparent or opaque; the latter are but seldom seen now, but were formerly held in high esteem, and may occasionally be met with in old collections. They were made by a process of double decomposition of certain chemical salts, with the addition of size or gelatine, to give a body to the injection. Mr. Thomas Davies, in his work "On the preparation and mounting of microscopic objects," gives the following formula for the yellow injecting fluids, which will be of service, should the student wish to try his hand at making this form of injection:—

Acetate of lead	380 grains.
Bichromate of potash ..	152 „
Size	8 ounces.

The lead salt is to be dissolved in the warm size, and the bichromate of potash, in powder, is added gradually with a good shaking-up between each addition; or

Acetate of lead	190 grains.
Chromate of potash ..	100 „
Size	4 ounces.

The first of these produces the deepest colour, and is most generally used. For a white injection, the second salt must be varied thus:—

Acetate of lead	190 grains.
Carbonate of potash ..	83 „
Size	4 ounces.

In these combinations, a double decomposition takes place, and an insoluble salt is thrown down, a chromate of lead being formed in the case of the yellow, and a carbonate of lead, or "white lead," formed in the latter. These injections, when made and while still warm, must be strained through fine flannel, to reduce the particles to such a degree of minuteness as to enter the capillary

system without blocking it, and causing extravasation. Preparations injected in this manner can only be examined under the microscope, as are other opaque objects.

By far the most interesting and instructive are transparent injections; at the same time, they are more easily accomplished. Dr. L. S. Beale, in his book "How to Work with the Microscope," in giving directions for this operation, recommends the following formula for a blue transparent injection; and as the author has frequently employed it, he can testify to the successful results he has always obtained, and to the facility with which good transparent injections can be made with it. This blue injecting fluid is formed in this manner:—

Price's glycerine	2 oz. by measure.
Tincture of sesquichloride of iron..	10 drops.
Ferrocyanide of potassium ..	3 grains.
Strong hydrochloric acid	3 drops.
Water	1 ounce.

Mix the tincture of iron with one ounce of the glycerine and the ferrocyanide of potassium, first dissolved in a little water, with the other ounce. These solutions are to be mixed together very gradually in a bottle. *The iron solution must be added to the ferrocyanide of potassium.* Lastly, the water and the hydrochloric acid are to be added; sometimes a little alcohol, about two drachms, may be added to the mixture. About six ounces should be made for a rat or small guinea pig. If properly made, this injecting fluid should be entirely free from particles or even granules, and look more like a blue stain than the result of a double decomposition. The author makes it a little more acid than does Dr. Beale, as in subsequent staining with the ammonia carminate, unless the blue injection is distinctly acid, all its colour becomes degraded or almost lost after a few years, by the alkalinity of the carmine stain.

Red transparent injections are also recommended as

instructive and pretty; the formula given by Dr. Beale will be found to work well, but as it contains gelatine, both subject and solution must be kept warm :—

Pure carmine	1	drachm.
Liquor ammonia	2	drachms.
Glacial acetic acid	1	drachm 26 minims.
Solution of gelatine (1 part to 6 of water)	2	ounces.
Water	$1\frac{1}{2}$	ounce.

Dissolve the carmine in the ammonia and water, and filter if necessary. To this add an ounce and a half of the hot solution of gelatine, and mix thoroughly. With the remaining half ounce of gelatine solution, mix the acetic acid, and then drop this little by little into the carmine solution, stirring briskly during the whole time. Great care is required in mixing this injection that no precipitation of the carmine takes place by putting in the acid in too large quantities at a time; and therefore, while mixing, a little should be examined under the microscope, and the addition of the acid solution discontinued in time, should signs of precipitation appear. In carrying out this process, there is one condition not to be overlooked, and which, if neglected, will only end in failure, or, at the best, only partial injection. The animal must be freshly killed, and injection commenced before *rigor mortis* sets in, as the rigidity of the coats of the capillaries will prevent that smooth flow of the injecting fluid, which is conducive to a satisfactory result. This fact being always borne in mind, we may pass on to a description of the apparatus employed. Presuming the student to be minus the elaborate appliances of a fully equipped physiological laboratory, we must point out two simple methods by either of which the injection may be thrown into the circulatory system, beginning with that most usually recommended :—

An Injecting Syringe.—This is a syringe of brass, of about two ounces capacity, having a well-fitted piston

INJECTING SYRINGE.

working easily, but not too freely, the handle of which is formed of a ring sufficiently large to allow the thumb to pass through; and two rings in which the fingers are placed.

Nozzles.—These, when in use, are firmly fixed to the end of the syringe, and are generally supplied in three or more sizes, to accommodate the varying calibres of the vessels to be injected. The apparatus employed by the author renders the operation so much less fatiguing than when conducted by the syringe, that it will commend itself, after a fair trial, as one less likely to end in failure from too much force being applied by the hand in syringe injecting. This arrangement consists in having two Woolf's bottles placed on a shelf, about five feet above the laboratory table; one of these is partly filled with the injection fluid, and the other with warm weak solution of table salt in water. A bent glass tube, inserted through a cork in one of the necks of the bottle, passing to the bottom, acts as a syphon. Two lengths of indiarubber tubing, and of sufficient length to reach the table, should be well washed out, to free them from the many particles which will be found in all new tubing. In the free end of each length of tubing is fastened such a stop-cock as are generally supplied with the syringe. A tube may now be attached to the short end of each glass tube. The tube may be now sucked by the mouth, till the fluid in the bottle begins to flow down the tube. When it runs out, the stop-cock must be turned off. This is done to prevent any air getting ingress to the vessels, as that contingency must ever be guarded against as fraught with danger to the success of the operation. We have now two tubes filled with fluid, and constituting a column whose weight is sufficient to overcome the resistance of the vessels. It becomes necessary now to commence operations at the heart; but before doing so sundry accessory appliances should be provided. A nozzle should be selected as near the size of the *aorta* as possible. The nozzles have two projecting spurs, the

use of which we shall see presently. A ligature needle should be constructed as follows:—Flatten the end of a stout piece of copper wire, and having drilled a hole in it, polish the end very smooth, and fix it in a wooden handle. Have ready some fine twine, or stout thread; now insert the nozzle into the left ventricle, having previously filled it with salt solution. When the tube has passed within the arch of the aorta, put the ligature needle, threaded, under the vessel; draw the thread through and remove the needle, and tie the tip of the nozzle in the vessel, and fasten the ligature to the spurs before mentioned. This prevents the nozzle from slipping out of position. Now insert the stop-cock belonging to the salt solution into the nozzle, taking care no air gets between, and turn the tap, when the diminishing depth of the solution in the bottle will tell whether it is flowing through the vessels to wash them out. When sufficient has flowed through, it is only necessary to transfer the stop-cock to the blue injection, when, if things have gone well, the exposed tissues will almost immediately become coloured. This flow may be left uninterrupted for such a time as may be deemed enough to permit the capillary system to be thoroughly permeated. This plan answers equally well for the carmine gelatine injection; only everything must be kept warm to prevent the gelatine setting. The author prefers this plan to that with the syringe, as the constant renewal of the fluid with this tends to rupture the vessel, unless great care is observed; and another objection arises from the fatigue to the hand in keeping up a constant and steady pressure during the process. In this plan the nozzle being fixed once and for all, and without any strain, not such a danger as a rupture presents itself, while the steady flow of a five-feet column of fluid is sufficient without hand pressure. There are methods given in the books included in the appendix, by which insects, mollusca, &c., may be injected, and to which the student may be

referred. Before dismissing this subject, it may be advisable to say a few words in reference to the mounting of injections. The medium for mounting, in most cases, is Canada balsam, and although this is easy of application, it robs an injected preparation of half its beauty, and nearly the whole of its instructiveness. We see, 'tis true, an exquisite network of vessels, but look in vain for those important parts the vessels are there to nourish; these are blotted out and obliterated by the balsam. The mountant, therefore, should be one which allows the relations of vessels to their immediate substructure to be clearly seen and studied. Such a medium as glycerine solutions of varying densities should be chosen; that solution selected which shows these relations the best. The mucous coat of the stomach and intestines, however beautifully and completely injected, lose half their value as instructive agents when their glandular and villous elements are stamped out so completely as they are in the greater number of specimens we come across.

DIATOM MOUNTING.

Diatomaceæ.—There is no branch of microscopical study which possesses greater charms for those who take up its pursuit, and once get within the ring-fence of its fascination, than the study of the diversified forms of the diatomaceæ. The insatiable appetite of the diatomist grows by what it feeds upon, and luckily there is no stint; for this class of microscopical life is universal in its distribution. It may be met with recent as well as fossil, in fresh water as well as in the sea; there is scarcely a spot in nature where it cannot be found. And it is necessary

here to direct the student to the localities where they may be found, and to say a few words relative to their collection and preparation. Diatoms may be obtained all the year round, but are most plentiful in the spring and summer. Each kind has its favourite habitat; some prefer growing on the algæ in the sea, and on its shore, others have to be searched for in the muddy crevices of the rock. One variety, the *melosira*, is common in marshes, where they may be recognised as brownish masses. Others may be sought for on the surface of mud. Oyster beds are very productive of diatoms, and the stomachs of these succulent bivalves furnish admirable hunting grounds, abounding as they do in many varieties. In fact, diatoms are so ubiquitous, that search where you will, it will be strange if they are not met with, so that the student who is bent upon making a collection, need never be at a loss for material wherewith to gratify his desire. In examining ponds, especially those found in marshes, where the water is brackish, like those at Swanscombe, in Kent, a most abundant gathering may be made, and yielding a great number of varieties, as many as forty-three having been identified in one dip. They are generally found floating on the water as yellowish brown masses, or lying on the surface of the mud. In sunshiny weather they will be found floating, because, like other vegetables, they give off oxygen in sunlight, which floats them to the surface; and should a breeze blow over the surface of the pond, this raft of diatoms may be driven to leeward, where they may be gathered tolerably free from mud and other impurities. The instrument which is used to collect them may be anything of a spoon shape, such an one as resembles a housemaid's dust-pan in miniature, broad and shallow in front, and deep behind. This, attached to a long handle, may be gently slid under the edge of the raft, which will then float into the deeper end, and thence be transferred to a collecting bottle, and labelled with the locality in

which the collection was made. Such an instrument will also be found of great use in skimming the surface of mud where diatoms are suspected. Having made the collection, one of the most troublesome operations in which microscopists can engage, is that of freeing the diatoms from impurities; hence various plans have been adopted to accomplish this with as little difficuity as possible. If they adhere to fresh water plants, or to marine algæ, and are free from mud, rinsing them off in water, to which a small quantity of hydrochloric, or acetic acid, has been added, will detach them in a tolerably clean condition; but where they are mixed with mud and other contaminations, nothing but repeated washings and decantings will separate them. The author has practised with much success a plan suggested by Dr. H. Munro, in the journal of the Microscopical Society for 1855, page 242, in which he says, "I first boil the deposit in strong hydrochloric acid for five or ten minutes, then allow it to subside, pour off all the acid, and by a few washings get as much of it away as possible. Then treat the deposit in the same way with strong nitric acid, washing the deposit by repeated washings, to get rid of the remaining acid. When this is done, I then separate the diatoms, according to their different gravities, by allowing them to pass through a column of water, in the following manner:—I take a long glass tube about four feet long and half-an-inch bore. At the bottom of this tube is fixed a stop-cock, to enable me to let out any of the diatoms during any stage of the process. Having nearly filled this tube with distilled water, I pour in my deposits washed free from the acids. I watch the deposit as it falls slowly and gradually down the tube. In about a quarter of an hour many of the larger forms will have descended to the bottom of the tube. By turning the tap at the bottom of the tube, I let out a drop of the mixture on a slide;" and so he proceeds, waiting a longer interval between each turning of the tap to allow

of the lighter forms settling. In this manner, they are tolerably well sorted into sizes. The only weak point about this plan is that it merely separates bodies of the same specific gravity, so that foreign matter is not eliminated entirely, but sinks with the diatoms. The larger specimens which descend with the grains of sand in the first fall may be easily picked out from the unwelcome neighbours, by plans to be detailed later on.

Professor Bailey, in an article in Silliman's Journal, recommends the following plan, which also, like the preceding, he found to give excellent results, he says:—"Dissolve out the lime compounds, if present, by means of nitric acid or hydrochloric acid, then wash and filter; put the moist contents of the filter into a porcelain capsule, with enough strong sulphuric acid to make the whole a fluid mass. Heat the capsule over a spirit lamp until the organic matters are all charred, and continue the heat until strong acid fumes are evolved. Keep the capsule hot, and in minute portions at a time, add finely-powdered chlorate of potash. If the acid is hot enough to give off fumes, the chlorate will be immediately decomposed without the accumulation of explosive gases, and it will exert so powerful an oxidising action, that in a few moments a carbonaceous material as black as ink will become clean and colourless. Nothing will now remain to be done but to wash off the acid, which is best done by the addition of water, and repeated decantations. I would advise that the material thus cleaned should not be dried, but should be kept in bottles with a little alcohol, which prevents their felting together, and does not allow of the byssoid plants, which often develop in water. It is necessary to caution those not familiar with chemistry against using the chlorate of potash with sulphuric acid in any other way than that above directed, as violent and dangerous explosions might result. The process as given above is perfectly safe, and very effective."

If the student does not choose the mode of separation prescribed by Dr. H. Munro, he may, by using a long dipping tube, arrive at somewhat the same results. The washed and cleaned deposit may be stirred up in a cylindrical glass measure, and dippings of the first sediment examined under the microscope. He will then probably find large grains of sand mixed with the larger specimens of diatoms. Dr. Carpenter recommends that these diatoms should be pushed on one side, by means of a bristle inserted in a wooden handle. If this plan is adopted, great gentleness must be used, as even, with such a comparatively delicate instrument, a rough touch will end in the fracture of the fragile frustule. Professor Redfern suggested the use of split hairs, such as may often be found in an old shaving brush, for this purpose. These do not appear split till they are pressed on the diatom, when they open and grip it like fairy forceps; but even here care must be employed, lest a fracture of the valve ensues. Mr. William Borrer, Junr., devised an ingenious arrangement for picking out, and arranging diatoms, and, which, under his hands, was attended with great success. It may be described as a collar of cork fitting on to the lower end of a low power objective, and through which a needle is thrust obliquely. To the end of the needle, one of the barbed lancets of a bee's sting, or what is even better, the bristle from a rat's tail is cemented. By adjusting the point of this in such a manner as to be in focus at nearly the same time as the diatom, it can be brought down on to it gently, and, as it adheres to the barb, it can be raised and transferred to a clean slide. Some mounters of diatoms acquire an unusual dexterity in cleaning, selecting and mounting these minute frustules, as evidenced by the slides of A. C. Cole and L. Hardman, and the still more marvellous achievements of Herr Möller, but the student will hardly be likely to aspire to the perfection attained by these men, but will generally

be content if he can mount diatoms cleanly and suitably for his own satisfaction. This brings us to the subject of mounting them after the preliminary processes just touched upon. Of late years a search has been made, and is still being prosecuted, with the view of finding a suitable medium in which to mount diatoms. Balsam, which satisfied generations of bygone diatomists, stands a chance of being relegated to a back row, while newer agents take its place. It is preferred by some as a good mountant, still; the only fault found with it being its low index of refraction, when used with the high power immersion lenses, which have come into vogue during the last few years; but taken all round, and except for comparative tests it is far the most convenient medium. If we were to deal with the various media brought to the front in the battle of diatom resolution, it would swell this small *brochure* to unwieldy dimensions, and be of little utility to the beginner. Gum styrax finds support from some, and a preparation introduced by Dr. Van Heurck, of Antwerp, of which styrax forms a good proportion, gives very good results, and the same time is convenient. Its mode of use is simple. A small portion being removed from the bottle, is warmed slightly, and put on a warm cover glass, which is inverted, and gently allowed to fall on the slide on which the diatoms are placed. Not too much heat must be employed, or annoying air bubbles will form; and not too much pressure used, or the diatoms will be broken. This preparation is very dark to look at in the mass, but as only a very thin layer exists between the cover and the slide, it may be regarded as sufficiently colourless for this and for many other mounting purposes, and remains unchanged in character for years. Mr. H. Morland, one of our best English authorities on this subject, states, in a paper recently read at the Quekett Microscopical Club, that "American Styrax hardens just like balsam, but when heated becomes extremely fluid. Having a high refractive index, it is particularly useful for the finer diatoms. On

account of its fluidity when heated, I should be inclined to confine the use of this medium to the smaller diatoms, lest the cover should, during the process of mounting, sink down on the larger forms, and crush them."

It may be as well, in treating of this subject, to make a passing mention of the medium employed by Mr. J. W. Stephenson, who finds a valuable resolving power in a solution of phosphorus in bisulphide of carbon; but, until the student advances sufficiently far in his microscopical studies as to devote himself exclusively to this branch, it would be advisable not to play with so highly inflammable a solution. Professor H. L. Smith and Mr. W. C. Meates have devised media, in which sulphur, arsenic, and bromine are compounded, and although they give admirable mounts when first set up, yet after a short time crystallisation gradually sets in, and spoils the slide, so that, taking these drawbacks to the use of these media into consideration, it will be as well to be content, for the present, with balsam and Van Heurck's preparation. Of course, it will be always remembered that this Manual is only for amateurs, who are not expected to rush into the thick of the fight about markings and difficult resolution; they will find that out for themselves in time. Associated with diatoms, the collector will often find desmids; but these may be readily distinguished from diatoms by their bright emerald-green colour. The desmidieæ are a family of fresh-water algæ, and are amongst the most beautiful objects which can attract the young microscopist, whether from their transparent green colour or the great diversity of their forms. Unlike the diatoms, the desmid grows only in fresh water, and prefers a quiet pool in an open and exposed situation; and although they may be found in streams, it will be useless for the collector to seek them unless the stream is a very slowly running one. Small shallow pools in boggy ground are most likely spots to find them in; and they may be easily collected by the shallow spoon recommended

for the collection of diatoms, if, like them, they are spread on the surface of a muddy bottom. When they grow on the stems of aquatic plants, these may be gathered bodily, and transferred to a glass bottle of water. If they are scattered through the water, they may be collected by a small muslin ring net, to be described later on, and the net turned inside out in the bottle of water, when the desmids will be washed off; and if this is placed in the sunlight on reaching home, by the next morning, the desmids will be found congregated at that side of the bottle nearest the light, tolerably free from foreign contamination.

Mounting media for desmids, which will preserve their beautiful colours, are still to be invented. There are only two which seem to act in this direction fairly well. One is to add camphor water to that in which they have been accustomed to grow, when they may be mounted in it. This plan answers as well as any, and is to be preferred. The other mode is the employment of a preservative solution recommended by Mr. Thwaites:—Distilled water sixteen parts, alcohol one part, and a few drops of creosote; stir in a small quantity of chalk, and filter. Mix the liquid with an equal part of camphor water, and, before using, make a cone of stout blotting paper the size of an ordinary thimble, and filter as much as required for one mounting on the slides, to which the desmids to be mounted have been previously placed. Mr. Topping recommends as a good preservative for these, and also for delicate algæ generally, a solution of one part of acetate of alumina to four parts of distilled water; but, with all these good mounting media, the colour after a time disappears from the endochrome, and only the outer form remains to tell of the primitive beauty of the desmid. We cannot take leave of these lovely forms of microscopic plant life, without calling attention to one species, which will always excite interest, whether we regards its animal-like movements or the violent vicissitudes through which, index, it is particularly useful for the finer diatoms. On

during some part of its life history, it has to pass. The plant to which we allude may often be found in the metal gutters of buildings, where they are subjected to such extreme heat, that the hand brought incautiously in contact with the metal in the height of summer, is speedily withdrawn; or it may be found on the alpine snows in the depth of winter, where it is better known as "red snow" than by its botanical name of *Protococcus pluvialis*. The dried up powder in a metal gutter will often afford a crop when placed in water and deposited near a window, when, in a day or two, that side of the water near the light will be found swarming with pale green moving bodies. A dip may be made with the tube, and the drop examined under the microscope, in a shallow cell, when an immense number of pear-shaped bodies, having a red spot somewhere about them, will be seen in rapid locomotion. No means, whereby this motion is accomplished, are visible, but can be rendered so by the administration of a most minute dose of a solution of osmic acid allowed to absorb itself under the cover glass, by putting the drop of solution on one side of it, and a small piece of blotting paper on the other, which, as it abstracts the water, allows the osmic acid solution to take its place. This almost instantly kills the protococcus, and soon it begins to assume a darkened appearance, when from its small end, two long slender filaments will be seen, which, lashing the water, propel the alga along. The osmic acid may now be withdrawn by the same method by which it was introduced, distilled water taking its place; there will always be enough of the osmic acid left in the remaining fluid to act as a preservative, when the cover glass may be cemented down.

The consideration of the foregoing seems, by a natural sequence, to lead on the collection of infusorial life; an examination of which forms such an attractive feature in microscopical investigation.

INFUSORIA.

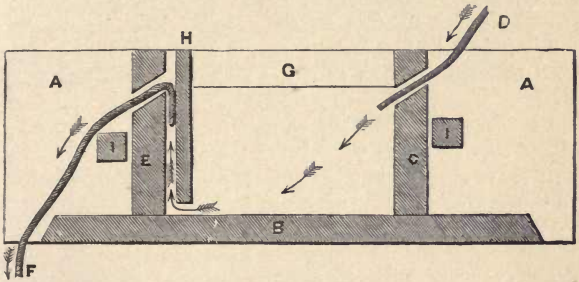
To pursue this branch of his studies with convenience and comfort, the student will require sundry accessory apparatus, which will be explained here. It is hard to stand by a promising pool, and gaze with longing eyes at an aquatic plant, which you know is loaded with the life you are seeking to capture, but without the means of getting at it; so to obviate this difficulty, *a collecting stick* must be obtained. This which looks like an ordinary walking-stick, but artfully concealing joints in its interior by which it can be lengthened, may be carried out by the student whenever he takes his walks abroad in the neighbourhood of likely ponds. But the stick is not enough: he must have something to take his infusorial life home with him. Nothing answers this purpose so well as the screw-top York bottles, obtainable of most chemists. One or two of these carried in the pocket *almost* completes, with the stick, the *armamentarium* of the collector, but not quite. Sold with the stick is a metal ring, having a screw thread within it which fits the screw on the York bottle. Another screw on its side enables it to be firmly and securely attached to the stick, so that when the bottle is screwed into the ring, and the ring is screwed to stick, and the joints of the stick are joined, we have a piece of apparatus as long as a fishing rod, but stiffer, which can reach out to the middle of any ordinary pool. But the student will naturally say, "How will this get up the water plants I want to examine?" but let him look in his collecting kit, and he ought to find a sharp sickle-shaped hook, which will screw into the end of the stick when the screw carrying the bottle is removed. This taking the place of the ring, can be thrust under water, and the coveted plant reaped, and brought to the surface. He may be anxious to know if he has got what he expected to find

without the necessity of carrying home a useless burden in an abundance of weed. For this purpose, various home-made appliances in the shape of square and round, clear glass bottles, have been impounded; but the handiest, as it is also the most efficient, of these appliances may be constructed thus: Take two pieces of clear patent plate glass about three and a half inches square, grind their edges, and cement with marine glue between them, at the side and bottom, some slips of stout plate glass. This forms a strong shallow form of trough, in which aquatic plants may be placed for examination by the pocket lens. The parallel sides of the glass trough favouring the examination, such portions of the plant may be selected for further inspection, and placed in separate tubes, for it is not a good plan to huddle everything collected in one stock bottle, as many beginners are apt to do. It must be always remembered that overcrowding in any shape is not only detrimental to the well-being of animal existence, but even fatal to its ultimate success. In addition to the collecting stick, bottle, ring and hook, a muslin ring net, and a shallow spoon, as recommended in diatom and desmid collecting, should be added, and the pond-hunter's kit contains all absolutely necessary. Arriving at home with the spoils of the hunt, they will need further examination, distribution into separate glass tanks, and placing in the light of a north window if possible, the direct rays of the sun being prejudicial by overheating the small quantity of water, and by favouring the inordinate growth of the confervæ. Before leaving this subject, it will be not without advantage to say that many collectors and breeders of infusoria seem to consider the pleasant summer afternoon is the only and proper time for these collections. No greater mistake can be made, for though they may be rewarded by the capture of forms of great beauty, by confining their operations to this one season of the year, they leave uncaptured many of these forms in

their early and transitional stages. If they would increase their knowledge of the life history of many of these infusoria that charm them in the summer, they should visit those same ponds in the winter, and beneath the snow and ice they would find an abundant harvest of material, and an unwonted interest in developing the ova of many of the fresh-water fauna. For this purpose, a slide, capable of being transferred to the stage of the microscope at any time without disturbing the object under observation, must be added to the stock of accessory apparatus. Such a slide is known as *a growing slide*. Many forms have been devised, but that which the author prefers, not only because it is his own invention, but more especially for the many advantages arising from its peculiar construction in affording members of the infusorial family the conditions necessary to their healthy development may be now described. It often happens that, in examining a gathering from some aquatic source, an organism is met with, about which the observer would desire to know more; but to transfer it from his slide to one of the growing slides in ordinary use, would most probably result in its loss or destruction. The slide now about to be described, is designed to supersede the use of the glass slip generally used for this purpose, so that should such an organism present itself, it is not necessary to disturb it, but simply to insert an inlet and an outlet thread of darning cotton in the openings at the sides of the cell, attaching the inlet thread to a small reservoir of water, a few inches above the microscope stage, when a continuous current, favoured by the capillary attraction of the cotton threads, is made to pass through the cell. Any number of these slides may be constructed by the microscopist himself, for, by having half a dozen or so at hand, it becomes an easy matter to put any one having an object of interest on it aside for future examination. They are designed with a view of being put away in the grooves of a slide box.

Such plain directions may here be given for making them, that, aided by the illustration, no difficulty ought to be experienced in their construction.

The slide consists of the usual glass slip AA (3 in. × 1 in.), having a narrow ledge of glass B (about $\frac{1}{8}$ inch wide), and extending nearly its whole length), cemented to its lower border with marine glue; to this is cemented at right angles a strip of thin covering-glass C, about $\frac{1}{4}$ inch wide, and about $1\frac{1}{8}$ inch from the end of the slide, having a narrow channel cut through it for the passage of an intake thread D. A similar strip E, having a like cut through it for the passage of an outlet thread F, is cemented at the same distance from the opposite end of the slide. In this condition, the slide being filled with water to the level of G, any current coming in through the intake thread



Growing Slide.

D, would pass directly across the top of the water in the cell, and pass out by the outlet thread F, and organisms near the bottom of the cell would not be benefited by a change of water; therefore, cement a very narrow slip, H, of the same covering-glass as before to the inner side of the outlet end of the cell, commencing at the top of the slide, and extending to very nearly the bottom, so as to leave about $\frac{1}{16}$ inch between E and H. If the intake thread is con-

nected with a bottle of water placed above the level of the slide, water entering by the intake thread will pass in a diagonal direction from D to the left and bottom of the cell, where the influence of the suction, set up by the siphon-like action of the outlet thread makes itself felt, and there is a regular current in the direction of the arrows.

The front of the cell is formed of a piece of covering-glass of $1\frac{1}{2}$ inch by $\frac{5}{8}$, and two small, square blocks of glass, cemented on each side, will hold this covering-glass sufficiently firm to prevent it sliding on the organism and crushing it.

Such a growing slide will hold about 1 drachm of water, and taking the rate of the drops from outlet thread as about one per minute, the whole of the water in the cell is changed once in an hour, while, at the same time, the current is not sufficiently strong to carry away more than the finest and lightest bodies. It allows of fair observation with a $\frac{1}{4}$ inch objective, and, if desired, could be made with thinner glass, so that a $\frac{1}{6}$ inch or $\frac{1}{8}$ inch might be used.

In using this growing slide, any reservoir of water placed slightly above the level of the stage will answer the purpose of supply; but the author has had a support fixed to the moveable stage of his microscope, which carries a small bottle of water at a suitable height, and thus the water supply and the growing slide are made to move simultaneously without any disturbance of the object during its examination; in this manner he was enabled to carry on continuously for two months the observation of the development of *Corethra plumicornis*, when the successive changes in its organisation were carefully noted and *drawn*.

This brings us to the consideration of another very important phase in the young microscopists career, *i.e.*, the power of taking records of such objects of interest which may be new to him, for if drawings of these are carefully

made and submitted to other observers more advanced in microscopical observation, many a mare's nest is demolished, while new and previously unobserved facts are preserved.

DELINEATION OF OBJECTS.

Drawings from microscopical observation are made by means of an optical arrangement placed over the eye-piece of the microscope, and called a *camera lucida*. Many forms of this instrument are in vogue, but the main principle on which they are formed is the same, that is, the rays of light from the object is reflected at an angle, and the object made to appear as if projected on the drawing-paper, when its outlines and details can be drawn with a pencil. Some are made to be used when the microscope is in a vertical position, which is the most convenient method for the examination and drawing of infusorial life, unless confined in a shallow trough, when the microscope may be placed horizontally, and a *camera lucida* made upon the following principle may be used: the cap of the eye-piece being removed, a collar of brass may be substituted, carrying a reflecting mirror at an angle of 45° with the optical axis of the microscope; some are made to carry a small mirror the size of the pupil of the eye, others of larger extent, but with all it is very necessary to keep the eye in a fixed position till the drawing is finished. The plan of proceeding is as follows: the microscope being inclined till it comes to a horizontal position, the cap of the eye-piece removed and the *camera lucida* put on, it must then be determined to what extent the drawing is to be enlarged, if it is to be the size it is magnified, the same distance must be maintained between

the *camera lucida* and the paper, as exists between the *camera lucida* and the object magnified; but by increasing the distance between the *camera lucida* and the paper, the magnification of the drawing can be increased proportionately; thus, if the eye-piece carrying the *camera lucida* be projected beyond the edge of the table, the drawing can be made large enough for a diagram, but the pencil must be elongated to reach the paper on the floor, and the hand and eye must be very steady to successfully accomplish such an enlarged drawing. A better plan is to invert a small simple microscope carrying the object to be drawn, and on illuminating this in a dark room, cast its image on drawing paper, then in proportion as the microscope is elevated above the table, so will the enlargement be increased, and not only this, but the natural colours of the object may be painted in. An exceedingly simple and efficient *camera lucida* may be constructed by a cap fitting on to the eye-piece, carrying a square of cover glass at an angle of 45°; this, if broken, can be easily replaced; its upper surface reflects the image of the object, which, on looking through the thin glass, appears on the drawing paper beneath, and if this paper is fixed to a drawing board with the usual pins, the drawing may be discontinued and resumed at leisure, a few trials being sufficient to bring about a readjustment of the image to the outlines.

The student seeking for information on other forms of this very necesary instrument, will find them fully dealt with in Dr. Carpenter's work. In drawing with the *camera lucida*, it is all important to so arrange two lamps that, while one dimly but clearly illuminates the object, the other should throw the fullest light on the pencil; but the apportionment had better be left to individual requirements; it, however, aids to have the cedar-wood drawing pencil freshly cut, that the light wood shining up through the under surface of the reflector, may be a guide to its

whereabouts. Many beautiful drawings, full of detail, have been made by simply copying the image as seen in the field of the microscope by dropping into the eye-piece, in the focus of the eye-glass, a circle of glass ruled in equal squares, and copying the image as a map is copied; but whatever method be employed, the drawing entails a vast amount of careful labour and a great expenditure of time; also, however scrupulous the draughtsman may be, however unbiassed he intends to be, errors may creep in, and therefore photo-micrography, or the art of photographing microscopical enlargements, comes in to ensure complete veracity with a saving of labour. There is no great difficulty in carrying out the process, but photo-micrography, to be successful, must be approached by easy stages; it is of very little use to plunge *in medias res* by employing high power objectives before the comparatively simple routine of taking a photograph with a low power has been mastered; and simple as this routine is, there are difficulties which present themselves at every turn, but by careful attention and experience they can be overcome, and the operator rewarded with a satisfactory result.

PHOTO-MICROGRAPHY.

In photo-micrography we have the converse of micro-photography, which is applied to the making of such small photographs as to require the aid of the microscope to render them visible, while photo-micrography is applied to the portrayal of microscopic specimens by photographic enlargement. The reason for mentioning this distinction is to correct a tendency very generally prevalent that

these terms are mutually convertible, and may be used with indifference.

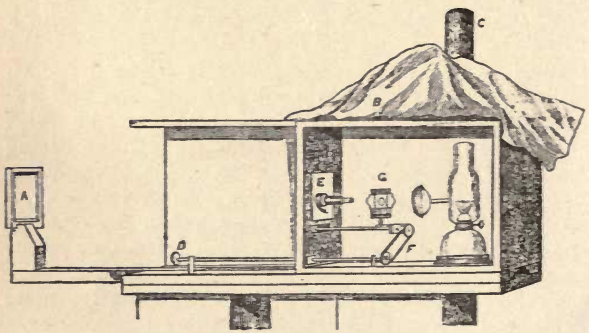
Photo-micrography has been practised many years; but the author has not thought it desirable to swell the dimensions of this Manual by giving a full history of its rise and progress, but for those who may feel interested in working out this history, he may say that Dr. Lionel S. Beale, in his valuable book "How to work with the microscope," 1865, states that Mr. Dancer, of Manchester, produced photographs of microscopic objects about 1840. From that time forward various workers in this field of science, both in America and England, have taken it up with increasing satisfaction in the success of their results. It is to be regretted that the records of their labours are too scattered up and down throughout the literature of science to permit their being searched up, and bringing within the compass of this contribution; but a sufficient bibliography has been appended at the end which will be found to supply the needs of those who feel a desire to acquaint themselves with the labours of others.

In all the processes described by these various authors, the microscope has been employed in conjunction with the camera, and beautiful results have been attained thereby; but while fully admitting this, the author has never felt satisfied with the constricted field of view produced by the microscope tube; therefore, in the method introduced now, he discards the use of the microscope entirely; the advantages claimed for this being—first, the field of view is only limited by the size of the sensitive plate employed, a great range of amplification is obtained by varying the distance between the objective employed and the sensitive plate, and an amplification suitable to the subject can be easily selected. A second advantage derivable from this arrangement is in the ability to see your image and arrange it according to the part you desire to photograph;

this can be done on the ordinary ground focusing glass of the camera, but when the image is thrown on to a white ground, as it is in this apparatus, it is more easy to select any particular portion, and to focus it roughly. This method is only suitable for photographing transparent objects, but as it is more immediately designed for the delineation of histological subjects, nothing can exceed its adaptability to do this in comfort and ease.

Before proceeding further, it may make these remarks more readily understood if we briefly describe the photographic arrangement employed. Imagine an oblong lidless box, laid on its side, and securely screwed to one end of a base board, two inches in thickness and two and a half feet in length; the upper central part of this base board, about one inch in thickness, is made to slide in a dovetailed groove—the end of this sliding part carries the holders for the plates employed—this holder being an ordinary printing frame, in which photographic negatives are printed from. The size of the holder may be varied according to the amplification required, and this sliding holder can diminish, or greatly extend the magnification as may be desired. The upper side of this box has an oblong opening cut in it, over which a tin chimney is fixed, thus allowing the lamp to approach or recede from the object stage as may be desirable. Another opening is made in that side of the box which faces the plate holder and central with it; this opening is closed by a movable brass plate, having an adaptor with the standard microscopical screw soldered into it: this permits any objective with the Society's screw-gauge being employed. Below this plate a support carrying the moveable stage is fixed to the side of the box, this stage being moved backwards and forwards by a long micrometer screw, the subject to be photographed is made to approach or recede from the objective till a sharp image is thrown on the screen; the best focusing screen is made by covering an

old glass plate of any of the standard sizes intended to be employed, by gumming on a sheet of the smoothest white paper; this enables the operator to arrange the object according to his judgment, and permits of a certain amount of rough focusing. The finest focusing must be arrived at by other means; another plate of plain glass put into the holder, having *fine* lines drawn a short interval apart with a writing diamond on the surface of it which faces the objective, is looked at from the back through an ordinary eye-piece, or a photographic focusing glass, and when the details of the objects are seen sharply at the same time as the fine lines, a sharply defined image will be thrown on the sensitive gelatine



A—Frame for receiving sensitive plate and focusing screen, B—Black velvet curtain for shutting in the light. C—Chimney. D—Handle of focusing rod. E—Sliding plate for changing objectives. F—Endless band acting on fine adjustment of G—The object carrier.

plate, which it is intended shall occupy in the holder the place now occupied by the plain glass.

The light is derived from a microscopical lamp, burning the purest paraffin oil, in which is dissolved a lump of camphor of the size of a walnut to the ordinary reser-

voirful; this whitens the flame and renders it more actinic; a plano-convex lens, with the convex side towards the flame, serves to concentrate the light on the object. A curtain of black velvet falls over the front of this arrangement, shutting all light in, and a shutter cuts off the rays coming through the objective till all is ready for them to fall on the sensitive plate, during the proper time of exposure. Now this is, in brief, a short description of the apparatus and it next demands a few directions relative to the *modus operandi* practised.

The lamp having been lighted and placed inside the box, is allowed to burn some short time before commencing work, that the wood and brass work may be warmed up; for, if this is not done, the expansion of these after you have focused your object will result in a blurred picture. Your microscopical slide may next be placed in position, and its projected image arranged on the white paper surface of the focusing screen. You will soon learn to see by the character of this image, whether it will require a long or a short exposure; whether it reveals much detail, or is marked by its entire absence; or whether its colour favours photography or not. If the object or its image possesses detail of a fairly well-marked character, and the object is not too thick, you may calculate upon getting a successful result if you give the *correct* exposure. And now you are brought face to face with the first of the difficulties.

A most valuable paper on photo-micrography by lamplight, may be read in Nos. 13, 14, and 15 of the "British Journal of Photography," for 1885, written by an accomplished American optician, Mr. W. H. Walmsley, and which we strongly advise those interested in this subject to read. It is most succinctly and clearly written, and contains the whole gist of the subject. In the matter of normal exposures by lamplight he gives the following table, which may be taken as approximating to the

correct, but may require modification by each operator. When using

$1\frac{1}{2}$	in. objective	3—45 seconds.
$\frac{2}{3}$	„ „	7—90 „
$\frac{4}{10}$	„ „	$\frac{1}{2}$—3 minutes.
$\frac{1}{5}$	„ „	2—7 „
$\frac{1}{10}$	„ „	4—10 „

In working out this branch of photography, the Author has had no help from books or friends, and his experience tallies exactly with that of Mr. Walmsley, and he can fully endorse all he recommends.

Having selected your object and focussed your image, cut off the rays of light from the screen and shut in every vestige of light. Now being in perfect darkness, light your non-actinic lamp; these are sold of various patterns, but may not be actinically safe, as may be found to your cost. That recommended is a paraffin lamp with all light blocked out through the reservoir, an argand wick and a tubular chimney well coated inside and out with Thomas's ruby varnish; this will afford abundance of safe light at eighteen inches distance. By this light take your sensitive plate from its box, and put it into the focusing frame, when its gelatine surface ought to be in exact register with the fine diamond markings by which you get the true focus; then open your objective, and let the image of your preparation fall on the gelatine plate for the number of seconds or minutes given in Mr. Walmsley's table, after which shut off the light again, and proceed to develop. We need not say much on this subject, because it has been very fully treated in all the elementary books on photography. Some operators prefer the ferrous oxalate developer, while others adopt that which is known as the alkaline pyrogallic acid. We have used both, and although having got very good negatives with the pyrogallic acid, prefer the ferrous oxalate, because it does not not stain the fingers. As some may like the simplicity

as well as the cleanliness of this developer, and as this Manual may be perused by those to whom the subject may be entirely new, it may be as well to give the formula for making it. Having made two *saturated* solutions, one of neutral oxalate of potash, and another of sulphate of iron, add them together in the proportion of one part of iron to three parts of oxalate of potash, *taking care always to add the iron to the potash*, it makes a dark sherry coloured mixture; if it is feared that the plate has been slightly over exposed, add a few drops of a ten per cent. solution of bromide of potassium, which acting as a restrainer, keeps back the too rapid development of the image. Keep up the development till the general ground of the plate does not transmit any light and all detail is out in the image; then give the plate a good rinse in a pail of water and place it in the fixing bath, the solution in which, is made by dissolving four ounces of hyposulphite of soda in a pint of water; let the plate remain in this some minutes after the bromide of silver, which has not been acted upon by the light, and which remains yellow, is dissolved out, when it may be removed and washed copiously in running water, dried spontaneously and varnished, when it may be printed from.

Further details of this process may be obtained from the many small and excellent treatises dealing with ordinary photography, and therefore the space of this book need not be occupied in describing what may easily be read in them; but the process by means of this photographic arrangement reduces photo-micrography to the greatest simplicity, and enables anyone to delineate sections or membranous tissues with the greatest ease. When powers higher than one-fifth of an inch focus are used, it might be desirable to have a more powerful light than that afforded by the paraffin and camphor, but, as a rule, that will be found sufficient for such powers as we are most likely to employ, especially if the

edge of the wick be presented towards the object. A further use to which this apparatus may be put, is as a projection microscope. A screen of tracing paper taking the place of the focusing screen, and of any convenient dimensions, may be made to receive the image in a darkened room, when several persons can examine it with as great facility as if looking at it through the microscope. Another use may be found for it, a plane mirror silvered on its face and fixed at an angle of 45°, with the course of the rays as they pass through the objective are thrown down on a sheet of drawing paper, and thus the image may be traced and even coloured in its natural colours.

THE MICROSCOPE AT THE SEASIDE.

One of the great advantages of the microscope is the ready means it affords the microscopist of carrying on his pursuits, whether for recreation or instruction, wherever he may find himself located, and now that several of our best makers construct their instruments in a portable form, no difficulty arises to prevent the microscope being his constant companion in his holiday trips. No field of interesting research so abounds in objects well worthy his attention as the sea-side, and here the student may readily make himself familiar with marine fauna and flora to any extent, for the rocks and the algæ will furnish him with a variety of diatoms to be studied in their living state, while on a calm warm day the surface of the

sea will be found teeming with the embryonic forms of many of the crustacea, &c. The rock caverns, at spring tides, will well repay the search for different kinds of sponges, and various species of the *Ascidia*. The ordinary collecting kits of the pond hunter will suffice for many of the localities he will visit. In the rock pools, deep down, he will find treasures in the shape of forests of living *Polyzoa*, whose feathery forms he will soon learn to distinguish; if these are attached to loose fragments of rock or pebble, they may be transferred bodily to a jar of clean sea water, and will there live sufficiently long to be examined for several days; but they are delicate creatures and do not brook confinement for long without drooping. The jar recommended to be employed is such as used by confectioners for their sweets and fancy biscuits. In use, it should be covered by a glass plate, to check evaporation as much as possible, and if one growing frond of the green sea lettuce (*Ulva latissima*), attached to a clean stone be put into the water, sufficient oxygen will be evolved by its growth to maintain the polyzoa in a tolerably healthy condition for some time. The jar should be placed where light can fall upon it freely, but it must not be exposed to *direct* sunlight, as the small bulk of water would be heated thereby, and anything above the normal temperature is prejudicial to the polyps. A needful piece of accessory apparatus in marine research is a fine muslin tow-net. It is made in the shape of a funnel, about one foot in diameter at its wide end, and securely attached to a ring of *brass* wire varnished with shellac; if an iron ring is used, it is apt to become soon rusty; so the author prefers the brass, as in that case the muslin net lasts longer. This tow-net may be either towed after a boat as it is rowed along, or it may be attached to a stick, and the surface of the water skimmed from the side of the boat, a plan to be preferred, because the wash of the boat at its stern often scatters those forms which had been

attracted to the surface by the sun. Whichever plan be adopted, the net must be occasionally raised and drained, and then turned inside out in a pail of clean sea water, when its contents will be washed off ready for examination on the return home. In examining marine objects in sea water, the careful student will always use a plate-glass stage, on the ordinary stage of his microscope, to protect his brass work from the corroding action of sea salts. His ingenuity will also enable him, from pieces of glass cemented together securely with marine glue, to construct any special tank he may find necessary for the examination of the polyps—that is, if he has not gone already prepared with such, which is always advisable. Beautiful and dazzling as these polyps are in their living actions, the student may desire to preserve the remembrance of them in their dead forms, and to put them up for his cabinet is one of the most troublesome processes he will find himself engaged in, but at the same time one so satisfactory, if attended with success, that all are advised to try it. Various plans have been suggested and tried. Thus, some recommend a very dilute solution of osmic acid; this answers the purpose tolerably well, if most gradually and stealthily introduced into the trough while the tentacles are expanded. It has, however, the drawback of slightly blackening the creatures, which detracts from their beauty. A weak solution of bichloride of mercury, of about two parts of the bichloride to one thousand parts of water, introduced in the same way, has been successful in the hands of some mounters; but the best results in the author's experience have been derived from the use of whiskey in this manner. A piece of glass tubing has been melted over a spirit lamp, and drawn to a capillary point; this has been broken off, so as to leave a very minute opening in it. If this tube be now filled with whiskey, and inserted by the side of the trough holding the expanded polyps, the spirit will trickle in very small drops down the side of the trough, and become so

gradually diffused through the sea water that the polyps becoming accustomed to its blissful influence, succumb to it, and forget to draw in their tentacles. In mounting these as permanent preparations, nothing succeeds so well as a preservative fluid, as it answers for most marine objects, as glycerine and camphor water.

Amongst the many interesting microscopical objects to be examined at the sea-side are those tiny shells known as *oraminifera*, from the number of foramina which pierce their walls. They may be found in their living state attached to marine algæ, or in the oozy beds of a rock pool at the lowest tides. It has been stated by some naturalists that they float on the surface of a calm sea in sunny weather; whether that is generally true or only true in certain localities remains to be verified; but the author, without wishing to compare big things with little ones, from an extensive experience of their habits in an aquarium can testify to the fact that he has never seen them rise to a greater height than four inches from the bottom, and that by means of their pseudo-podia, as they clung to the front glass; they multiplied very fast, and could be collected in abundance from the sand. The foraminiferous shells collected at the sea-side, containing, as they do, the living animal, require cleaning; this is best done by boiling in strong potash solution, which dissolves the sarcode of the animals without injuring the shells; then, after repeated washings to clear away the alkali, they may be dried and picked out with a stiff bristle slightly moistened in the mouth, they may thus be transferred to the cell in which they are to be permanently mounted. As they require fixing to the bottom of the cell, and as gum solution is inadmissible in all dry mounts, the cell must be lightly painted with a varnish made of *genuine* asphaltum. For mounting the foraminifera on it, they merely require placing, and the temperature of the slide being slightly raised, the shells become, as it were, glued by their under

surfaces to the slide by the thin layer of asphaltum without its flowing up and over their sides, which frequently occurs with other black varnishes. If the recent foraminifera are placed in a very weak solution of hydrochloric acid, the shells, which are composed of carbonate of lime, are dissolved away from the enclosed sarcodic body, which, after washing, may be stained and mounted in the glycerine and camphor water mixture. The chalk of the cliffs abounds in these beautiful objects in a fossil condition, together with the testæ of different varieties of entomostraca, which can be liberated by the chalk being brushed under water in a hand basin, using a softish nail-brush, when by the gradual disintegration of the chalk, the foraminifera, &c., are separated without much damage, and can be picked out of the detritus and mounted. Some clays abound in foraminifera, and may be separated by making the clay thoroughly hot and plunging it into water, and then by repeated washing clear away all the fine particles till the sediment, consisting of sand and shells, remains; they require after this a great deal of careful cleaning, even with the potash solution, to get them quite clean. This is a troublesome process, and one which the student perhaps may not care to follow out, unless he feels inclined to take up the study of the foraminifera as a specialty, when he will find fuller particulars in the researches of Carpenter, Rupert Jones, and Parker, and others, who have made it their particular study. To those who would make themselves familiar with these interesting forms of microscopic life without the trouble entailed in the process just mentioned, the author would recommend a visit to the bins of some friendly dealer in Turkey sponge, when he may probably be favoured with a quart of the sand which drops from the sponges, the sand being sifted away through muslin sieves, will leave a choice residue of many forms of foraminifera, besides the small spines of *Spatangus* and the *Echinoidea* generally. The foraminifera may be picked out

with the bristle, or sometimes this residue may be sprinkled on the top of some water, the heavier particles of sand sink to the bottom, and the shells containing air float on the surface, and may be skimmed off and dried.

By chipping flints, especially those of a yellow colour, the silicified outlines of foraminiferous shells, principally *Textularia*, may often be found associated with sponge spicules and gemmules and the fossil sporangia of the desmidiaceæ, known as *Zanthidia*. To chip these flints they must be held on a solid block of iron, and struck with the sharp end of a hammer. If the blow brings off an irregular lump, the position of the flint must be changed, and a blow again given with the hammer; this must be repeated till the plane of cleavage is discovered, when the flint will break off in flakes. These may be placed in glycerine and examined; those containing any organic remains of interest may be stored away in spirit till they are mounted in balsam. Unless the student can gain access to a lapidary's wheel and slice his flints, it is better to buy them ready-mounted of those dealers in microscopical objects, who make this branch a speciality. Failing this, to examine such thin flakes as he can obtain by the above method.

THE MICROSCOPICAL SOCIETIES.

As an ounce of help is worth more than a pound of pity, so is the student likely to gain more insight into the use of the microscope and the making of preparations from that friendly gossip which always takes place when kindred spirits and congenial minds meet to talk over those subjects in which they take a common interest. Many little

difficulties may arise in his work, which all the books ever written will be useless in enabling him to surmount, when half an hour's chat with one who has encountered and overcome the same trials will put him quickly in the right path. With this view, it has been deemed advisable to introduce here a few particulars of those societies whose advantages to the microscopist, of whatever age and standing, are incalculable.

First and foremost in the encouragement of microscopical research stands the Royal Microscopical Society. Meeting in its own rooms at King's College, London, this society offers to its Fellows, amongst many advantages, a bi-monthly journal, containing a full report of all the latest advances in microscopical research, both British and foreign, with notes and illustrations of all the most modern improvements and additions to their favourite instrument and its accessory apparatus; also a copious bibliography of all recent writings bearing on microscopical science. Nine meetings are held during the winter and spring months, at eight o'clock on the second Wednesday of each month, at which papers are read, and objects and apparatus of interest are exhibited.

It has a very complete and extensive library of books bearing upon every branch of microscopical work, freely accessible to the inquiring student, for reference on the evenings of meeting, under the assistance of an energetic and amiable librarian, who is both able and willing to point out and procure whatever works the student wishes to consult. There are two meetings in the session, especially devoted to the exhibition of the latest objects of interest. These advantages, with the privilege of appending F.R.M.S. after your name, are acquired by the payment of two guineas as an annual subscription, with an entrance fee of two guineas the first year. This society alone is sufficient to meet all the wants of the young beginner in microscopical investigation; but there

is another which might almost be styled "*the nursery*," as many who begin their career in it generally find themselves, when they are "brought out," becoming Fellows of the parent society—it is the Quekett Microscopical Club. This meets at University College, in Gower Street, on the second and fourth Fridays in every month throughout the year. The meeting on the second Friday has been called the "gossip" night; members take their microscopes and some objects of interest, which are exhibited, and their mode of preparation explained. These evenings are most instructive to the tyro, for, however backward he may be in his efforts to go forward, he will meet with kind and ready help from all he comes across. All will evince a friendly desire to put him on the right road. The fourth Friday is devoted to the reading of papers by the members, and discussion thereon. The club possesses a very complete library, the books from which the members are at liberty to take home to study. It also has a cabinet of objects, a certain number of which the members may borrow for examination at home. In addition to these advantages for study, outdoor excursions for the collection of objects of natural history, botanical specimens and infusoria take place every Saturday afternoon fortnight during the summer months, and are generally accompanied by some of the veterans in the art of collecting and instructing. The club publishes an illustrated journal about every quarter, containing the papers read, and their discussion. The subscription to this club is ten shillings per annum, and without entrance fee, so bringing its advantages within the reach of even the youngest student. These are but two of the London societies open to the lover of the microscope. There are clubs founded on the basis of the Quekett Club, not only in the suburbs, as at Ealing, Brixton, Hackney and Croydon, but also in the provinces, and even in some of the colonies, which may be recommended as beneficial

centres with which the student might associate himself, and where, from the author's personal experience, he will meet with that aid and refreshing intercourse which not only encourages and helps, but stimulates to fresh exertion, where the members feel that they add to their own pleasures in communicating their knowledge to the earnest seeker after truth.

ODDS AND ENDS.

THE blow-fly's tongue has always been a favourite object with microscopists, whether fulfilling its mission as a test object for flatness of field of view, or simply on account of the beauty of its pseudo-tracheæ, but the blow-fly affords pleasure in many ways; as furnishing dissections it is unsurpassed, whether alive or dead it always affords interest, but these advantages are more readily supplied by the blow-fly in its recently hatched condition. Blow-flies, as the student may be doubtless aware, come from what are known in the fishing world as "gentles," which, after enjoying a short existence in this state, enshroud themselves in a brown case, and await that propitious season when, bursting their bonds, they issue as newly-fledged blow-flies. At this period of their lives they are as neat and clean in their persons as the most scrupulous microscopist could wish. Their internal viscera are at that moment free from all contamination of food, and readily takes any stain applied to it by reason of its newness. To obtain it in this state of cleanliness, the following plan must be adopted:—A hole should be made in a tin biscuit box, and a piece of glass tubing, about

half an inch in internal diameter, passed from this hole through a bung into a clean, dry, wide-mouthed bottle. The chrysalids of flies, together with the sand in which they are supplied, being put into the tin box, they hatch in time, and as they hatch, find their way into the glass bottle through the tube. Feed such a newly-hatched fly on syrup stained with cochineal, and on dissection the whole alimentary tract can be readily discerned by the colour. It is at this age that it will be found easier to lay out the proboscis in the usually accepted style, while the wings under an air-pump will often expand into bladders as imprisoned air separates the two surfaces when the pressure of the atmosphere is removed. While speaking of the air-pump, it may be mentioned that insects may have their tracheal system injected with carmine stain if they are placed in it under the receiver and the air alternately exhausted and let in two or three times in succession. The air being drawn out of the tubes during the first process, the carmine runs in to supply its place during the second. If the insects are colourless and transparent, the colour may be traced even up to the tips of the antennæ.

The *Demodex folliculorum* is a minute acarus, whose place is, however, scarcely defined by naturalists, but which derives considerable interest not only from its place of habitation, from which it can be easily procured, but also from the lively movements it exhibits when first captured. It varies in length from about one-fiftieth of an inch to one-hundreth. If the black spots which often adorn the face of adolescence be squeezed, a little speck of fatty matter exudes; it is only necessary to take this up with an oiled pin, and transfer it to a slide, on which has been placed a little castor-oil, and covered with a thin and therefore light glass cover. On slightly warming this, the fatty matter dissolves and clears up, showing the Demodex in great activity. If the epithelial scales, which help to

make up the bulk of the fatty matter, are not so abundant as to hide the acarid, the cover-glass may be cemented down, as the mite is too minute to be transferred to another slide.

Ciliary action in Mussel.—This is one of the most attractive phenomena that can engage the attention of the young microscopist. It is not the province of this Manual to enter into the explanation of this action, as that will be found in all works on elementary physiology, but directions may be supplied by which these interesting movements may be observed. The mussel being opened, a fringe-like expansion of the mantle is seen spreading itself as a lining over the inside of each shell, a piece of this may be snipped off with the scissors, and laid on the slide with some of the fluid out of the shell. A cover-glass may be placed on it, when it may be examined. The cilia will be found in rapid vibration, becoming slower after a time, when the nature of the action can be more readily seen and followed.

Circulation in the Frog's foot.—This again, like the last, always excites considerable interest wherever exhibited well. To do this the frog should be made as comfortable as circumstances permit, by being placed in a linen bag, kept wet, and well supported by the piece of apparatus known as a frog-plate. If the frog is comfortable, it does not try to move its foot about, which would seriously interfere with the view. The leg must not be tied in the mouth of the bag too tightly, or the circulation will be stopped. Two threads tied round two adjoining toes must be so fastened to the pins of the frog-plate, that the distended web may be over the aperture in the stage. If this tying is done carefully and neatly, the web may lie pretty evenly in the field; it will require an occasional moistening with cold water.

Circulation in the Characeæ and Anacharis, may be observed by placing portions of these plants in a trough, and as they are favourite exhibits with many micro-

scopists, it may be serviceable to the young beginner to know something of their nature and the habitat. The Characeæ are a family of aquatic plants which might be classed with the fresh water algæ, did not their reproductive organs tend to lift them to a higher grade. They are popularly known as "stoneworts," from the habit they exhibit, in common with many algæ, of attracting to themselves calcareous salts dissolved in the water they inhabit, and depositing it on their outer tissues. This occurs in some individuals to such an extent as to deprive them of that transparency which the microscopical observer wishes to utilise in studying the circulation of the sap. They may be found in many ponds which are exposed to plenty of sunlight, where they present the appearance of feathery whorls disposed at intervals round an elongated stem. At first sight it is hard to distinguish them from *Myriophyllum*, but on being landed with the hook on the collecting stick, it will be seen that in *Chara* the stem seems twisted, while in *Myriophyllum* it is straight. It is not desirable here to enter upon the botanical differences between these two plants, as these can be fully studied in works devoted to that subject. The circulation in *Chara* may be most easily seen in the terminal leaflets, which, being newer in growth, are likely to be freer from incrustations. *Anacharis alsinastrum*, the leaves of which, when slightly yellow, and older than the rest, show the circulatory movements of the protoplasmic contents of the cells, may be found in many ponds, although in many parts of the country it is disappearing. It is one of those plants which grow in water without being rooted, and in this way it keeps water containing infusoria and small animals in a healthy condition by reason of its evolving oxygen, and therefore capable of sustaining aquatic life. It thus serves the microscopist in a double capacity, affording him specimens in which circulation may be studied, and ministering to the wants

of those minute animalcules which, without it, would die of suffocation. *Vallisneria* also exhibits the phenomenon of the circulation, but it needs splitting into two layers to see it well. There are many other vegetables possessing *hairs* in which this circulation may be seen, but those mentioned above will be sufficient to give the student for his first essays. Should the circulation be languid or movement be entirely absent, by carrying the plant in a small bottle of water in the pocket for an hour or two, the dormant stream will wake up to activity.

It is often desirable in a mixed company of friends to abjure the strictly scientific attributes of the microscope, and to exhibit something to them which may be described as being pretty rather than scientific, and one experiment never fails to provoke exclamations of wonder from those who see it for the first time. Having carefully focussed a plain slide on the stage of the microscope, let someone look through the eyepiece, while a drop of spirits of camphor is run on the slide. At first nothing is seen, and the observer may be about, with evident chagrin on his countenance, to give up to the next, when a loud cry of "Oh! how beautiful!" cannot fail to draw the attention of all in the room. The spirit of camphor while fresh put on, does not show anything under a spot lens or a paraboloid, but as soon as the evaporation of the spirit causes the crystals to form, they show up suddenly as a silvery efflorescence, which is both startling and beautiful. Nothing exhibits the capabilities of a good spot lens so well as this. Another experiment may also be exhibited as an opaque object lighted by the bulls-eye condensing lens: place a drop of a solution of nitrate of silver in distilled water on the slide, and nicking a very small scrap of a bronze coin off with your penknife, wet this scrap with distilled water, and place it in the centre of the drop of silver solution. Immediately bright leaves of glistening silver dart out from the metal, forming not only an

attractive object at the time, but one which may be mounted permanently. Crystallisation is always watched with great interest, and is one of those subjects which the student should familiarise himself with, so that he may readily recognise the crystals of the various salts he may meet with in his investigations, always bearing in mind that outward influences modify shape in crystals as well as in animal life. Let him crystallise bichromate of potash from a plain aqueous solution on a slide, and compare it with another having a little gum arabic dissolved in it, and he might mistake them for different salts. Let him modify the temperature, and he will detect a slight change; but the most amazing changes may be produced from a solution of hippuric acid in absolute alcohol; such changes, that every slide showing a different aspect, might be mistaken for a separate salt. Let the solution be warm, the dipping rod and the slide warm. As soon as the solution touches the slide it diffuses itself, and becomes like a transparent film. Soon the moisture of the atmosphere begins to liberate the force which keeps back crystallisation, and the film is studded with round centres of commencing crystals; if now the slide be alternately warmed and cooled, a series of concentric circles will be formed round the primary dot; these increase till contact with adjoining systems meet, and crystallisation ceases; if instead of letting these circles increase, a sudden accession of moisture be supplied, as by quickly breathing on the slide, then rays of crystals start out from the edges of the circles, and a series of stars of varying size are formed. If this is done, accompanied by a moderately high temperature, the crystals form spirally, a higher temperature melts them, and their power of recrystallising is destroyed. Crystallisation, under the influence of different gases, considerably modifies the result; while crystallisation from an alcoholic solution in which arabin takes part will make the crystals take a

form totally unlike any other, and capable of being polarised as an opaque object. Enough has been said to show the wide scope for study which is included in the term crystallisation. When it may be thought desirable to mount any of these crystals, castor oil forms a good preservative; balsam causes them to break up in a most unsightly manner.

There is, perhaps, no movement observable under the microscope to which the student should direct his attention, more important than those movements of minute particles of matter, which are known as Brownian movements, from their being found by Dr. Robert Brown, to be associated with all minute particles in suspension in a fluid, whether they be organic or inorganic in their nature. Nothing is more likely to lead the student astray than to see these movements, and not be aware of their character, such as one would be led to imagine he had discovered living elements, where only physical causes are at work. With a view to familiarise him with their appearance, it is only necessary to rub a little camboge on a slide in some water, and on examining a thin layer of this with a quarter inch objective, the particles of camboge will be seen dancing round each other all over the field of view. The late Professor Stanley Jevons told the author of a very pretty way of exhibiting these phenomena. A shallow trough was to be made by uniting two square cover glasses by three of their edges, and a small quantity of *kaolin* mixed with water was to be placed in it, and allowed to subside till the boundary line between the surface of the deposited *kaolin* and the water was well defined. If now a small drop of solution of soap be allowed to run down one side of this trough and spread itself gently and without disturbance over the surface of the *kaolin*, although no active movements of its particles will take place directly, yet, in a short time, the whole surface will be in a state of lively vibration, due to the

Brownian movements being set up by the slight addition to the specific gravity of the water due to the soap. The movements are not attributable to evaporation, because they may often be observed in closed cavities, but may be influenced by electrical conditions, and in this direction we must seek for their origin.

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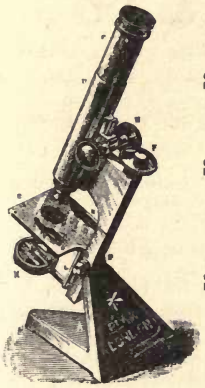

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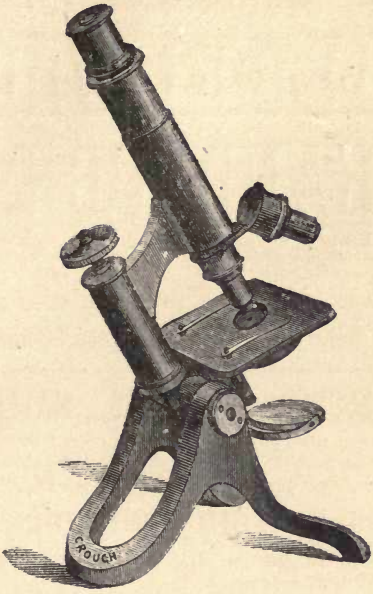

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